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CHEMICAL STUDIES OF CORN POLLEN.

II. Carbohydrates and Organic Bases.

By

SUGURU MIYAKE.

(*From Agricultural Chemical Laboratory, College of Agriculture, Hokkaido Imperial University, Sapporo*).

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I have previously published one part of the results (Miyake 1922) of my chemical studies of corn pollen, in which the general composition was determined, and i-inosite and phytosterol were isolated. Since then, I have studied the nature of the carbohydrates and organic bases of corn pollen, and the results of these investigations are to appear in this article.

Experimental Part.

Sample used.

The sample was collected in August 1922, by the method recorded in my previous report, from the same variety (Southern Horse Tooth) of Dent corn as in the former experiment, raised at Sapporo, Hokkaido, Japan.

A. Carbohydrates.

1. Carbohydrates of the alcohol extract.

5 kgs of the pollen thus obtained, were placed in a flask and digested with 80 per cent alcohol. From this mixture, the alcohol-extract was separated by filtration. The pollen residue was treated with a fresh portion of alcohol twice more. After mixing these extracts, the alcohol was removed by vacuum distillation from this solution.

The syrup produced was first purified with 80 per cent alcohol and then with 90 per cent. Thus about 50 gms of purified syrup were obtained, which showed the following reactions :

1) It indicated positively Molisch's reaction for carbohydrate.

2) It reduced Fehling's solution strongly.

3) The presence of the glucose group was confirmed by a saccharic acid test which was carried out by the Tollens and Gans method (1888). The quantity of silver in the purified salt was determined and found to be 50.85 per cent of this salt, which coincided well with the theoretical amount of 50.91 per cent in silver saccharate.

4) There was a difference between the content of reducing sugar in the syrup before and after invertion. This indicates the presence of non-reducing sugar.

5) The ketose group was detected by Selliwanoff's resorcin test (1887), which was carried out as follows :— the syrup was dissolved in a little water in a test tube, then a small quantity of resorcin and concentrated hydrochloric acid (1/4 in volume of the solution) were mixed with it and the mixture was heated. The characteristic fire-red colour appeared.

6) Then free ketose was detected by the Pinoff's test, that is, 10 cc. of 4 per cent ammonium molybdate solution and 10 cc. of the solution of the syrup were mixed in a test tube. After adding a little acetic acid, it was heated in a boiling water bath for 3 minutes. A blue colour appeared which indicated the presence of ketose.

7) Crystals of mucic acid were not produced by the oxidation of this syrup with nitric acid, this indicates the absence of the galactose group.

8) Mannose was not present, for mannosephenylhydrazone was not produced by the usual method, that is, by mixing the syrup with 20 cc. of water, adding 6 drops of phenylhydrazine and 3 drops of acetic acid. The mixture was stirred continuously,

and kept at room temperature, but it did not produce any crystals of mannosephenylhydrazone.

9) For the identification of glucose, the method recommended by Fischer (1890) was used. The syrup was dissolved in a little water and diphenylhydrazine in alcohol solution was added. By allowing the mixture to stand for several days, the diphenylhydrazone was crystallised out as small needles, which melted at 163° after recrystallisation.

10) The presence of the pentose group was confirmed by Tollen's deposit method (1896). The solution of the syrup was heated with an equal volume of concentrated hydrochloric acid and a little phloroglucin added. After standing for 3 minutes, the solution was then cooled and filtered. The precipitate formed was washed with water and then dissolved in 95 per cent alcohol. The alcoholic solution was coloured slightly violet and showed an absorption band in the yellow of the spectrum.

11) The presence of xylose could not be ascertained by Bertrand's method (1900). By this method, needle shaped crystals of cadmiumbromxylonate should be produced if the syrup contains xylose.

12) Neither could the presence of arabinose be verified by the method of Neuberg (1902). Though the needle crystals were produced, they proved to be crystals of glucose-diphenylhydrazone, after determination of the melting point.

13) The improved method of K. Kondo and K. Oshima was used for the detection of the methylpentose group, but the characteristic absorption band of methylfurfurolphloroglucide could not be observed.

14) Though, by the method of Schulze the isolation of the crystals of sucrose was attempted, the results proved unsuccessful. The syrup, however, exhibited the characteristic colour reaction of sucrose by the method proposed lately by Kryz (1922). The solution of the syrup was treated with 1 cc. of a saturated solution of $NiSO_4(NH_4)_2SO_46H_2O$, and then a few drops

of concentrated sulphuric acid were added to it. By boiling, it turned yellow and finally red.

15) The osazone test was carried out by Fischer's method (1884). 2 gms of syrup, 3 gms of sodium acetate, 2 gms phenylhydrazine hydrochloride and 20 cc. of water were mixed in a test tube and heated in a boiling water bath for one hour with frequent stirring. After the stated time, the solution was cooled and the crystals were collected on filter paper. The osazone obtained was then treated with hot water. The osazone soluble in hot water could not be identified because its quantity was too small for examination. The osazone insoluble in hot water was recrystallised from hot 95 per cent alcohol. After examination its melting point was found to be 204°. This osazone should be, therefore, that of fructose or glucose. The isolation of an osazone besides glucosazone from the alcohol solution of the crude osazone insoluble in hot water proved unsuccessful.

From the above tests, the following may be concluded:

The carbohydrates, contained in the alcohol extract, consist of reducing sugar and non-reducing sugar. As to reducing sugar, the presence of glucose and fructose was proved, but that of pentose was very doubtful. As to non-reducing sugar, the presence of cane sugar only was verified.

2. Carbohydrates of the water extract.

The pollen residue from the alcohol treatment was dried and extracted 3 times with 3 litres of water. The water extract thus obtained was cleared by filtration, and 95 per cent alcohol was then added to this solution until no more precipitate was produced. The precipitate formed, was separated by the centrifuge and washed first with 50 per cent, and then with 95 per cent alcohol, and dried in a desiccator over concentrated sulphuric acid. The precipitate thus obtained gave the reaction for carbohydrates and also for protein. The precipitate was dissolved again in water. The water solution was then heated in order to

coagulate the protein. The coagulum formed was removed by filtration and the solution was dialysed for purification. To this purified solution was then added 95 per cent alcohol until no more precipitates were produced. And this precipitate was washed successively with 50 per cent, 95 per cent, absolute alcohol and ether, then dried. The following reaction of precipitates was tested.

1) The water solution of this precipitate did not show any more the reaction of protein.

2) It turned its colour to brown by the addition of iodine solution.

3) It reduced Fehling's solution after hydrolysis with hydrochloric acid, but not before.

4) The absence of the ketose group was proved by the negative result of Seliwanoff's and Pinoff's test.

5) Neither could the reaction of pentose be observed.

6) It produced saccharic acid through the oxidation of the syrup with nitric acid.

7) The alcohol soluble osazone (m.p. 204°) was isolated from it by Fisher's method, after hydrolysis with hydrochloric acid.

From the above tests, it may be concluded that this white precipitate must be dextrin.

3. Carbohydrates of the insoluble part. (*Hemicellulose.*)

Since the presence of starch was verified by micro-chemical examination, 500 gms of the pollen, after extraction with alcohol and water, were placed in a flask and heated with water in a boiling water bath until the starch became gelatinised. When cooled to 60° malz extract was added and this mixture was kept at this temperature for 3 hours and then filtered. The same treatment was repeated twice more, and starch was removed completely. After washing the residue thoroughly with distilled water, it was extracted with a 0.2 per cent sodium hydroxide solution to remove

the protein. The residue thus obtained washed with distilled water to free it from protein and alkalies. It was then placed in a porcelain jar with 3 litres of 5 per cent sulphuric acid and heated on a boiling water bath for about 10 hours, and then filtered. The filtrate thus treated was neutralized with calcium carbonate and filtered again. The solution obtained was concentrated by evaporation with a small quantity of calcium carbonate on a boiling water bath, and filtered from time to time as soon as the preciptate was formed.

The syrup produced was purified with 80 per cent and then with 95 per cent alcohol.

The syrup exhibited only the following reactions for carbohydrates : — the Bertrandt's test for xylose, and the saccharic acid test for the glucose group.

So, it may be safely concluded that this syrup contains glucose and xylose. Therefore, the hemicellulose of the corn pollen consists of these sugars.

Summary.

The carbohydrates of the corn pollen consist of starch, dextrin, sucrose, glucose, fructose, and hemicellulose which is composed of glucose and xylose. The presence of free pentose is very doubtful.

B. Organic bases.

The syrup, gained from the pollen by extraction with 80 per cent alcohol for the investigation of the carbohydrates as previously stated, was also used for the isolation of organic bases.

After the syrup was dissolved in water, the solution was first treated with normal lead acetate and then with the basic salts. The precipitates found were filtered off and the excess of lead was removed from the filtrate as sulphate. Sulphuric acid was added until its amount was reduced to 5 per cent and then

a concentrated aqueous solution of phosphotungstic acid was added until no more precipitates were formed. The precipitate was filtered and washed repeatedly with 5 per cent sulphuric acid.

The bases were again liberated from the phosphotungstates by means of baryta of fine powder. After the precipitates of barium phosphotungstate were removed, the alkaline solution was neutralized with nitric acid, and then silver nitrate solution was added.

1. Purine bases.

The silver salts of bases formed by the above treatment were filtered, washed and treated with ammonia. The insoluble part of these salts was treated with hydrochloric acid and the silver was removed as silver chloride. The residue, obtained by the evaporation of this filtrate, did not indicate the reaction of guanin.

From the soluble part of this silver salt, the silver was removed by means of hydrochloric acid. The filtrate was then evaporated to a dry state under low temperature and dissolved in a little water. Next, the saturated solution of picric acid was added to the solution. The yellow prismatic crystals were then formed. These crystals, after recrystallisation from acetic acid, exhibited the following properties which coincided with those of adenine picrate.

1) It melted at 281°

2) The crystals were dried at 98° and analysed with the following results.

0.1 gm. substance ; 26.2 cc. N at 18° 768 mm.
Calculated for adeninepicrate. N 30.78%
Found. N 30.59%

Thus, these crystals proved to be adeninpicrate. After the adeninpicrate was separated from the solution, the filtrate was acidified with nitric acid, and the excess of picric acid was extracted with ether and evaporated to a dry state. Though

this residue was tested for the reaction of xanthin and hypoxanthin, no results were obtained.

2. *Hexone bases and others.*

From the solution from which the purine bases were removed as silver salts, none of the hexone bases could be isolated by the usual method.

The filtrate, separated from the precipitates which were produced by addition of excess of silver nitrate and barium hydroxide for isolation of the hexone bases, was treated with hydrochloric acid and sulphuric acid to remove silver and barium. The filtrate thus obtained was then acidified with sulphuric acid, and phosphotungstic acid was added. The precipitate produced was decomposed with baryta, and barium sulphate was removed. Then the filtrate was acidified with hydrochloric acid and evaporated into a small volume under vacuum. The crystal were produced which dissolved easily in absolute alcohol and produced a compound with alcoholic aurichloric acid which melted at 140°.

Therefore, this crystal must be choline.

Summary.

The presence of adenine and choline, as organic bases of corn pollen, was proved.

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ON LETHAL TEMPERATURE OF PURE KOJI-DIASTASE IN AQUEOUS SOLUTION AND RECOVERY OF ITS ACTION AFTER HEATING.

By

KOJI MIYAKE AND MITSUJI ITO.

(From the Laboratory of Agricultural Chemistry, College of Agriculture, Hokkaido Imperial University, Sapporo.)

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In a previous report from our laboratory it was proved that any temperature lower than 140°C., when heat was applied for a moment did not kill Koji-diastase in aqueous solution, and that at 140°C. itw as entirely destroyed. It was also found that at a temperature lower than 100°C. Koji-diastase in aqueous solution still retained its action even when heated for one hour, while its power was destroyed by heating longer than 30 minutes at 115°C. and 5 minutes at 130°C.

It was furthermore proved that the diastatic power of Koji-extract when injured by heating at a temperature lower than lethal, recovered to some extent by preserving the extract at room temperature, but that the extract heated at lethal temperature having lost its action completely, never recovered its power of saccharification even by preserving it for long intervals of time. The degree of the recovery of its action after heating increased with the prolongation of the time for preservation while it decreased with the increase of time of heating. However it never attained to a power equal to that of the original state.

The results given in the previous paper are those of the study with Koji-extract, and not with Koji-diastase in pure form. The present paper embodies, consequently, the results obtained with Koji-diastase in pure form.

On Lethal Temperature.

The sample for the present investigation was made as follows : 820 grams of Koji made from polished rice, under the same conditions with that in the previous investigation, were taken. After addition of 1.45 liters of 20% alcohol in volume it was left for 43 hours at room temperature with frequent agitation. Then it was filtered and washed with 200 cc. of 20% alcohol. One liter of the filtrate was obtained. To the filtrate, absolute alcohol was added making it altogether 80% in volume, then Koji-diastase was precipitated. The precipitate was gathered on filter paper, washed with 80% alcohol and then purified by Lintner's method. The yield of Koji-diastase thus obtained was 2.803 grams (0.329%). After analysis it was found to be almost pure, being freed from ash. To the 0.056% solution of this Koji-diastase, 2% toluol was added making it antiseptic and it was then placed in a bottle at a temperature of 5–15°C. The solution thus obtained was neutral in reaction. At the same time 2% soluble starch solution with 2% toluol as an antiseptic was prepared. The starch solution thus prepared was also neutral in reaction.

The diastatic power of the solution in each experiment which was conducted to determine the lethal temperature of Koji-diastase, was measured by the same method as that described in the previous report.

The result obtained was as follows :

Result 1. Diastase solution heated at 25°C. for 120 minutes.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration		
		Control	Heated	Difference
Diastase solution alone	0	0.10	0.10	—
Diastase solution + starch	0	0.20	0.20	—
	1	3.50	3.50	0

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titation		
		Control	Heated	Difference
Diastase solution + starch	5	5.10	5.10	0
	15	6.35	6.35	0
	25	6.40	6.40	0
	50	6.60	6.60	0
	100	6.70	6.70	0

Note:—

1. Control:—the relative power of saccharification of the original solution.
2. Heated:—the relative power of saccharification of the heated solution.
3. Difference.—difference of power of saccharification between the control and the heated solution.

The above notes have been used through the whole experiment.

In this case a weakening influence upon the saccharifying power of Koji-diastase in pure form, caused by heating was not observed.

Result 2. Diastase solution heated at 40°C. for a moment, and 120 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration				
		Control	For a moment		For 120 minutes	
			Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—
Diastase solution + starch	0	0.20	0.20	—	0.20	—
	1	3.50	3.30	-0.20	3.20	-0.30
	5	5.10	5.00	-0.10	4.85	-0.25
	15	6.35	6.30	-0.05	6.10	-0.25
	25	6.40	6.35	-0.05	6.20	-0.20
	50	6.60	6.55	-0.05	6.40	-0.20
	100	6.70	6.70	0	6.55	-0.15

It will be seen from the above result, that there was already an injurious effect upon the diastatic power by heating at 40°C. for a moment, and that when the time for saccharification is more than 5 hours showed a tendency to diminish the difference between the control and the heated. This result well coincides with that of the previous report.

Result 3. Diastase solution heated at 55°C. for a moment, 30, and 120 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration						
		Control	For a moment		For 30 minutes		For 120 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—
Diastase solution + starch	0	0.20	0.20	—	0.20	—	0.20	—
	1	3.50	3.00	-0.50	1.90	-1.60	1.70	-1.80
	5	5.10	4.80	-0.30	3.10	-2.00	2.05	-3.05
	15	6.35	6.05	-0.30	3.30	-3.05	2.55	-3.80
	25	6.40	6.20	-0.20	3.45	-2.95	2.60	-3.80
	50	6.60	6.40	-0.20	3.70	-2.90	2.80	-3.80
	100	6.70	6.50	-0.20	4.05	-2.65	2.90	-3.80

The injurious effect on the diastatic power was observed even by heating at the so-called optimum temperature 55°C. for a moment. The prolongation of time for heating caused more injurious effect on the saccharifying power of the enzyme.

The difference found between the control and the heated solution showed an interesting phenomenon, gradually increased for a certain time interval and then it gradually diminished. This is not only confirmed in this experiment, but also in the experiment described in the previous paper.

Result 4. Diastase solution heated at 70°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration											
		Control	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—	0.10	—
Diastase solution + starch	0	0.15	0.15	—	0.15	—	0.15	—	0.15	—	0.15	—
	1	3.50	1.65	-1.85	0.85	-2.65	0.55	-2.95	0.40	-3.10	0.25	-3.25
	5	4.85	3.15	-1.70	2.75	-2.10	2.25	-2.60	1.55	-3.30	1.30	-3.55
	15	5.60	4.45	-1.15	4.35	-1.25	4.30	-1.30	4.05	-1.55	3.25	-2.35
	25	5.90	5.00	-0.90	4.90	-1.00	4.60	-1.30	4.35	-1.55	3.55	-2.35
	50	6.05	5.35	-0.70	5.25	-0.80	4.95	-1.10	4.65	-1.40	3.70	-2.35
	100	6.30	6.05	-0.25	5.80	-0.50	5.40	-0.90	5.00	-1.30	3.95	-2.35

In this case we observed almost similar results to those obtained in the previous report with respect to the injurious effect of heating upon the saccharification of diastase.

Result 5. Diastase solution heated at 85°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in c.c. of $KMnO_4$ solution used for titration											
		Control	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—	0.10	—
Diastase solution + starch	0	0.30	0.30	—	0.30	—	0.30	—	0.30	—	0.30	—
	1	3.60	1.35	-2.25	0.85	-2.75	0.60	-3.00	0.55	-3.05	0.40	-3.20
	5	5.00	3.80	-1.20	2.80	-2.20	2.45	-2.55	1.80	-3.20	1.35	-3.65
	15	5.95	4.80	-1.15	4.35	-1.60	3.70	-2.25	3.05	-2.90	2.65	-3.30
	25	6.10	4.95	-1.15	4.70	-1.40	3.90	-2.20	3.30	-2.80	2.80	-3.30
	50	6.20	5.35	-0.85	4.95	-1.25	4.05	-2.15	3.50	-2.70	3.05	-3.15
	100	6.30	5.50	-0.80	5.20	-1.10	4.40	-0.90	3.70	-2.60	3.25	-3.05

The action of the diastase was greatly weakened by heating at this temperature even for a moment, but not yet totally destroyed even when heated for 60 minutes.

Result 6. Diastase solution heated at 100°C. for a moment, 5, 15, 30, 60, and 120 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration													
		Control	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes		For 120 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—	0.10	—	0.10	—
Diastase solution + starch	0	0.15	0.15	—	0.15	—	0.15	—	0.15	—	0.15	—	0.15	—
	1	3.50	1.20	-2.30	1.00	-2.50	0.65	-2.85	0.55	-2.95	0.15	3.35	0.15	-3.35
	5	4.85	3.10	-1.75	2.35	-2.50	1.70	-3.15	1.15	-3.70	0.40	4.45	0.15	-4.70
	15	5.75	4.80	-0.95	3.30	-2.45	3.20	-2.55	2.40	-3.35	0.80	-4.95	0.20	-5.55
	25	5.90	5.00	-0.90	4.50	-1.40	3.50	-2.40	2.70	-3.20	0.85	-5.05	0.20	-5.70
	50	6.15	5.40	-0.75	4.85	-1.30	3.75	-2.40	3.10	-3.05	1.00	-5.15	0.25	-5.90
	100	6.25	5.55	-0.70	5.00	-1.25	3.90	-2.35	3.40	-2.85	1.05	-5.20	0.25	-6.00

Though the action of Koji-diastase in pure form was considerably weakened by boiling, it was not yet totally destroyed even by heating at 100°C. for two hours.

Result 7. Diastase solution heated at 115°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration											
		Control	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes		
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—	0.10	—	
Diastase solution + starch	0	0.20	0.20	—	0.20	—	0.20	—	0.20	—	0.20	—	
	1	3.50	0.45	-3.05	0.40	-3.10	0.25	-3.25	0.20	-3.30	0.20	-3.30	
	5	4.95	2.10	-2.85	1.00	-3.95	0.45	-4.50	0.20	-4.75	0.20	-4.75	
	15	5.65	2.80	-2.85	1.60	-4.05	0.55	-5.10	0.25	-5.40	0.20	-5.45	
	25	5.95	3.10	-2.85	1.75	-4.20	0.65	-5.30	0.30	-5.65	0.20	-5.75	
	50	6.10	3.50	-2.60	2.45	-3.65	0.80	-5.30	0.35	-5.75	0.20	-5.90	
	100	6.25	3.85	-2.40	3.15	-3.10	1.15	-5.10	0.35	-5.90	0.20	-6.05	

The saccharifying action of Koji-diastase in pure form was not fully destroyed even by being heated at 115°C. for 30 minutes, though the action of Koji-extract was killed at the same temperature for the same time interval as proved in the previous report. The power of the Koji-diastase was completely lost when the period of heating was more than one hour.

Result 8. Diastase solution heated at 120°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration											
		Control	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes		
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—	0.10	—	
Diastase solution + starch	0	0.20	0.20	—	0.20	—	0.20	—	0.20	—	0.20	—	
	1	3.50	0.30	-3.20	0.30	-3.20	0.20	-3.30	0.20	-3.30	0.20	-3.30	
	5	4.95	0.55	-4.40	0.30	-4.65	0.20	-4.75	0.20	-4.75	0.20	-4.75	
	15	5.65	1.00	-4.65	0.35	-5.30	0.25	-5.40	0.20	-5.45	0.20	-5.45	
	25	5.95	1.45	-4.50	0.40	-5.55	0.25	-5.70	0.25	-5.70	0.20	-5.75	
	50	6.10	2.05	-4.05	0.55	-5.55	0.30	-5.80	0.30	-5.80	0.20	-5.90	
	100	6.25	2.35	-3.90	0.60	-5.65	0.35	-5.90	0.30	-5.95	0.20	-6.05	

The above result shows that the power of Koji-diastase in pure form was not completely destroyed even by being heated at 120°C. for 30 minutes. By being heated for 60 minutes it was entirely destroyed.

Result 9. Diastase solution heated at 125°C. for a moment, 5, 15, and 30 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration								
		Control	For a moment		For 5 minutes		For 15 minutes		For 30 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—
Diastase solution +starch	0	0.20	0.20	—	0.20	—	0.20	—	0.20	—
	1	3.50	0.25	-3.25	0.20	-3.30	0.20	-3.30	0.20	-3.30
	5	4.95	0.50	-4.45	0.20	-4.75	0.20	-4.75	0.20	-4.75
	15	5.65	0.80	-4.85	0.20	-5.45	0.20	-5.45	0.20	-5.45
	25	5.95	1.05	-4.90	0.25	-5.70	0.20	-5.75	0.20	-5.75
	50	6.10	1.30	-4.80	0.30	-5.80	0.25	-5.85	0.20	-5.90
	100	6.25	1.85	-4.40	0.25	-5.90	0.25	-6.00	0.20	-6.05

Koji-diastase in pure form lost its power completely upon being heated at 125°C. for 30 minutes.

Result 10. Diastase solution heated at 127.5°C. for a moment, 5, and 15 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration						
		Control	For a moment		For 5 minutes		For 15 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—
Diastase solution + starch	0	0.20	0.20	—	0.20	—	0.20	—
	1	3.50	0.20	-3.30	0.20	-3.30	0.20	-3.30
	5	4.95	0.20	-4.75	0.20	-4.75	0.20	-4.75
	15	5.65	0.25	-5.40	0.20	-5.45	0.20	-5.45
	25	5.95	0.30	-5.65	0.25	-5.70	0.20	-5.75
	50	6.10	0.50	-5.60	0.30	-5.80	0.20	-5.90
	100	6.25	0.75	-5.50	0.30	-5.90	0.25	-6.00

The saccharifying power of Koji-diastase was reduced almost to nil by being heated at 127.5°C. for 15 minutes.

Result 11. Diastase solution heated at 130°C. for a moment, 5, and 15 minutes, respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration.						
		Control	For a moment		For 5 minutes		For 15 minutes	
			Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—
Diastase solution + starch	0	0.20	0.20	—	0.20	—	0.20	—
	1	3.50	0.20	-3.30	0.20	-3.30	0.20	-3.30
	5	4.90	0.20	-4.70	0.20	-4.70	0.20	-4.70
	15	5.65	0.25	-5.40	0.25	-5.40	0.20	-5.45
	25	5.95	0.30	-5.65	0.25	-5.70	0.20	-5.75
	50	6.10	0.35	-5.75	0.30	-5.80	0.20	-5.90
	100	6.20	0.40	-5.80	0.30	-5.90	0.20	-6.00

The power of diastase in Koji-extract was destroyed completely by being heated at 130°C. for 5 minutes as proved in previous report, but that of pure diastase was not destroyed totally by being heated at the same temperature for the same time interval as seen in the above result. It was destroyed at this temperature when it was heated for 15 minutes.

Result 12. Diastase solution heated for a moment at 135°C., 137.5°C., 140°C. and 145°C., respectively.

	Time allowed for saccharification (Hr)	Volume in cc. of $KMnO_4$ solution used for titration								
		Control	At 135°C.		At 137.5°C.		At 140°C.		At 145°C.	
			Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
Diastase solution alone	0	0.10	0.10	—	0.10	—	0.10	—	0.10	—
Diastase solution +starch	0	0.20	0.20	—	0.20	—	0.20	—	0.20	—
	1	3.50	0.20	-3.30	0.20	-3.30	0.20	-3.30	0.20	-3.30
	5	4.85	0.20	-4.65	0.20	-4.65	0.20	-4.65	0.20	-4.65
	15	5.65	0.20	-5.45	0.20	-5.45	0.20	-5.45	0.20	-5.45
	25	5.95	0.25	-5.70	0.20	-5.75	0.20	-5.75	0.20	-5.75
	50	6.10	0.25	-5.85	0.25	-5.85	0.20	-5.90	0.20	-5.90
	100	6.25	0.30	-5.95	0.25	-6.00	0.20	-6.05	0.20	-6.05

Koji-diastase in pure form was not destroyed yet by being heated for a moment even at 137.5°C., while its action was completely destroyed at a temperature of 140°C. or over, as in the case of Koji-extract observed in the previous report.

From the above, the results may be summarized as follows:

The injurious effect upon the saccharifying power of Koji-diastase in pure form was not yet observed by heating at 25°C. for two hours, but a little injurious effect was observed by heating at 40°C. for a moment, and the injury became more pronounced

even when we heated it at the so-called optimum temperature 55°C. for 30 minutes.

Regarding the lethal temperature of Koji-diastase in pure form we had an almost similar result, though there was a slight difference, with that obtained in the previous experiment with Koji-extract. It was found that the action of Koji-diastase heated at 140°C. for a moment was entirely destroyed, and that, even when heated for one hour at a temperature lower than 100°C. Koji-diastase still retained its action, while it was destroyed by being heated at 115°C. or 120°C. for one hour, 125°C. for 30 minutes and 130°C. for 15 minutes, respectively.

B. On Recovery after Heating.

The sample for this experiment was the same as that used in the preceding experiment. After heating at various temperatures for various time intervals the sample was kept at room temperature for various time intervals as seen in the following tables.

The method of investigation for the existence and the degree of recovery of the saccharifying power was the same as that described in the second section of the previous report.

The time intervals allowed for saccharification were 25 hours in every case.

The results obtained were as follows.

Result 1. Diastase solution heated at 25°C. for 120 minutes.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration		
	Unheated	Heated	Difference
0	6.40	6.40	—
25	,,	6.40	0
50	,,	6.40	0
100	,,	6.40	0

Note:—

1. Unheated:—relative saccharifying power of the original diastase solution.
2. Heated:—relative saccharifying power of the heated diastase solution.
3. Difference:—difference of saccharifying power found between the control and diastase solution which was allowed to stand for various time intervals after heating. Difference represents, therefore, an existence of recovery of diastatic action and the degree of recovery at the same time.

In this case, there was no weakening influence caused by heating upon the diastatic action. Consequently no recovery function was observed.

Result 2. Diastase solution heated at 40°C. for a moment, and 120 minutes, respectively

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration				
	Unheated	For a moment		For 120 minutes	
		Heated	Difference	Heated	Difference
0	6.40	6.35	—	6.20	—
25	,,	6.35	0	6.25	+0.05
50	,,	6.35	0	6.25	+0.05
100	,,	6.35	0	6.25	+0.05

Recovery of diastatic function was observed a little in the case of heating for 120 minutes.

Result 3. Diastase solution heated at 55°C. for a moment, 30, and 120 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration						
	Unheated	For a moment		For 30 minutes		For 120 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference
0	6.40	6.20	—	3.45	—	2.60	—
25	,,	6.25	+0.05	3.55	+0.10	2.70	+0.10
50	,,	6.25	+0.05	3.55	+0.10	2.70	+0.10
100	,,	6.25	+0.05	3.55	+0.10	2.70	+0.10

In this case, a little difference between the control and the heated was observed, but the recovery did not bring the power of heated enzyme to that of the original enzyme in the limits of used time for standing, as was shown also in previous report.

Result 4. Diastase solution heated at 70°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration											
	Unheated	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	5.90	5.00	—	4.90	—	4.60	—	4.35	—	3.55	—
25	„	5.05	+0.05	5.00	+0.10	4.80	+0.20	4.55	+0.20	3.80	+0.25
50	„	5.05	+0.05	5.00	+0.10	4.85	+0.25	4.60	+0.25	3.85	+0.30
100	„	5.05	+0.05	5.00	+0.10	4.90	+0.30	4.65	+0.30	3.95	+0.40

The above result shows that recovery of enzymatic action clearly occurred in each of these cases, and the degree of recovery increased with the length of time allowed for standing.

Result 5. Diastase solution heated at 85°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration.										
	Unheated	For a momet		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	6.10	4.95	—	4.70	—	3.90	—	3.30	—	2.80	—
25	„	5.20	+0.25	5.00	+0.30	4.40	+0.50	3.70	+0.40	3.10	+0.30
50	„	5.25	+0.30	5.10	+0.40	4.45	+0.55	3.75	+0.45	3.25	+0.45
100	„	5.30	+0.35	5.15	+0.45	4.50	+0.60	3.85	+0.55	3.35	+0.55

From the result, recovery of enzymatic action was clearly observed in each of these cases, especially in the case of heating for 15 minutes.

Result 6. Diastase solution heated at 100°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration											
	Unheated	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	5.90	5.00	—	4.50	—	3.50	—	2.70	—	0.85	—
25	„	5.25	+0.25	4.75	+0.25	3.55	+0.10	2.75	+0.05	0.85	0
50	„	5.25	+0.25	4.80	+0.30	3.55	+0.20	2.85	+0.15	0.85	0
00	„	5.25	+0.25	4.80	+0.30	3.60	+0.25	2.90	+0.20	0.95	+0.05

Recovery in action of the enzyme was also observed in this case, and the degree of recovery was increased to the case in which the enzyme was heated for 5 minutes, then decreased gradually according to increase of time of heating.

Result 7. Diastase solution heated at 115°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration										
	Unheated	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	5.95	3.10	—	1.75	—	0.65	—	0.30	—	0.20	—
25	„	3.25	+0.15	2.35	+0.60	0.70	+0.05	0.35	+0.05	0.20	0
50	„	3.25	+0.15	2.35	+0.60	0.70	+0.05	0.35	+0.05	0.20	0
100	„	3.30	+0.20	2.35	+0.60	0.70	+0.05	0.35	+0.05	0.20	0

There was a recovery of enzymatic action still observed in the cases heated less than 30 minutes, but not in the case heated for one hour.

Result 8. Diastase solution heated at 120°C. for a moment, 5, 15, 30, and 60 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration											
	Unheated	For a moment		For 5 minutes		For 15 minutes		For 30 minutes		For 60 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	5.95	1.45	—	0.40	—	0.25	—	0.25	—	0.20	—
25	,,	1.60	+0.15	0.50	+0.10	0.30	+0.05	0.25	0	0.20	0
50	,,	1.65	+0.20	0.50	+0.10	0.30	+0.05	0.25	0	0.20	0
100	,,	1.70	+0.25	0.50	+0.10	0.30	+0.05	0.25	0	0.20	0
225	,,	1.70	+0.25	0.50	+0.10	0.30	+0.05	0.25	0	0.20	0
1200	,,	1.75	+0.30	0.60	+0.20	0.35	+0.10	0.25	0	0.20	0

In this case, recovery of enzymatic action was still observed a little, but was not observed in the cases of heating for more than 30 minutes.

Result 9. Diastase solution heated at 125°C. for a moment, 5, 15, and 30 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in c.c. of $KMnO_4$ solution used for titration.								
	Unheated	For a moment		For 5 minutes		For 15 minutes		For 30 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	5.95	1.05	—	0.25	—	0.20	—	0.20	—
25	,,	1.15	+0.10	0.30	+0.05	0.20	0	0.20	0
50	,,	1.15	+0.10	0.30	+0.05	0.20	0	0.20	0
100	,,	1.20	+0.15	0.30	+0.05	0.20	0	0.20	0
888	,,	1.25	+0.20	0.40	+0.15	0.25	+0.05	0.20	0

A recovery of enzymatic action was still observed, but not observed in the case of heating for 30 minutes.

Result 10. Diastase solution heated at 127.5°C. for a moment, 5, and 15 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration						
	Unheated	For a moment		For 5 minutes		For 15 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference
0	5.95	0.30	—	0.25	—	0.20	—
20	„	0.55	+0.25	0.25	0	0.20	0
50	„	0.55	+0.25	0.25	0	0.20	0
100	„	0.55	+0.25	0.30	+0.05	0.20	0
888	„	0.80	+0.50	0.30	+0.05	0.20	0

A recovery of enzymatic action was not observed in the case of heating for 15 minutes while a little recovery was observed in other cases.

Result 11. Diastase solution heated at 130°C. for a moment, 5, and 15 minutes, respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration						
	Unheated	For a moment		For 5 minutes		For 15 minutes	
		Heated	Difference	Heated	Difference	Heated	Difference
0	5.95	0.30	—	0.25	—	0.20	—
25	„	0.35	+0.05	0.25	0	0.20	0
50	„	0.35	+0.05	0.25	0	0.20	0
100	„	0.35	+0.05	0.25	0	0.20	0
225	„	0.40	+0.10	0.30	+0.05	0.20	0
270	„	0.40	+0.10	0.30	+0.05	0.20	0

A recovery of enzymatic action was scarcely observed in the case of heating for 5 minutes. It was not observed in the case of heating for 15 minutes. The result shows almost complete similarity to that obtained in the previous experiment with respect to a recovery of the action of Koji-extract.

Result 12. Diastase solution heated for a moment at 135°C., 137.5°C., 140°C., and 145°C., respectively.

Time allowed for standing after heating (Hr)	Volume in cc. of $KMnO_4$ solution used for titration								
	Unheated	At 135°C.		At 137.5°C.		At 140°C.		At 145°C.	
		Heated	Difference	Heated	Difference	Heated	Difference	Heated	Difference
0	5.97	0.25	—	0.20	—	0.20	—	0.20	—
25	„	0.25	0	0.20	0	0.20	0	0.20	0
50	„	0.25	0	0.20	0	0.20	0	0.20	0
100	„	0.25	0	0.20	0	0.20	0	0.20	0
270	„	0.30	+0.05	0.20	0	0.20	0	0.20	0

Koji-diastase in pure form heated at 135°C. for a moment scarcely had the power of recovering its action after being heated, but in other cases in which the enzyme was heated at 137.5°C., 140°C., and 145°C. respectively no more recovering action was observed. This fact shows that the enzyme solution which has been weakened by being heated at a temperature lower than lethal has the power of recovering its action, while the enzyme destroyed by heating has no power of recovery.

From the above ,results which coincide well with those obtained with Koji-extract, it may be stated that the enzymatic power of Koji-diastase in pure form injured by heating at a temperature lower than lethal, may recover to some extent by preserving it at room temperature. But the diastase heated at lethal temperature and having lost its action completely, never recovers its saccharifying power even when preserved for a long time.

In previous investigations on the same subject with Koji-extract, we assumed that the recovery of enzymatic action after heating, is caused by the recovery of action by the weakened enzyme, but not caused by the production of new enzyme from the mother substance present in the extract. This assumption is based on the fact that the temperature at which the enzyme is totally destroyed and at which the recovery ceases, strictly coincides. In this experiment we observed another fact which emphasizes our above assumption. The heating of diastatic solution caused the coagulation of protein like substance in a turbid state reducing its action greatly according to the degree of turbidity while the coagulum was dissolved gradually and a recovery of its action occurred gradually during the preservation of the solution.

Further investigations on this relation between turbidity and the power of the enzyme solution will be reported elsewhere in future.

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ON THE DISPERSITY AND THE SURFACE TENSION OF BLOOD SERUM.

By

TETSUTARO TADOKORO.

(*From the Biochemical Laboratory, College of Agriculture, Hokkaido Imperial University, Sapporo, Japan.*)

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Blood serum is an emulsoid which is composed of the polyphases of fibrinogen, globulin, albumin and of lipoids. The dispersity and the surface tension of the colloidal system are governed by the presence of the small quantity of mineral salts which correspond to one tenth of the colloid contents.

The study on the dispersity of blood serum is very important in serological research, as we believe the „Traube's theory" i. e. the neutrality of toxin-antitoxin is conceived as a process of the colloidal aggregation, which is caused by the adsorption phenomenon. So the specificity of the immune reaction is not caused by the presence of the definite chemical haptophore group but depends upon the definite condition of surface tension, cohesion and electric charge which are caused by the action of antigen (Dold 1922). Eisler (1923) proved that the dispersity of protein solution which bears an antibody is decreased by the addition of precipitin (refer Kraus and Pribram's report 1905). The so called Zammittest (Ascoli 1921) is the agglutination of micrococcus melitensis in milk. Therefore the dispersity and the surface tension of milk are changed by the addition of bacillus emulsion whereas the surface of a capillar is raised. The higher sensibility of Sachs-Georgi's test (used for the control of the Wassermann's reaction) is caused by the change of surface tension of alcohol extract in the presence of cholesterine (Epstein and Paul 1921). Ascoli and Izar's Meiostagmin reaction (1910) is the detection

by means of a stalagmometer of the reduced surface tension which is caused by the mixing of antigen and antibody. From these facts, it is easily understood that the determination of the dispersity and of the surface tension of blood serum has a great importance in serological study.

Until now the reports concerning this problem have been very scarce and I refer only briefly to them. Erdmann (1913) examined the surface tension of blood serum with his modified pipette which is an improvement on Traube's stalagmometer (1910). He stated that a small amount of acid causes a pronounced change of surface tension of blood serum and proves Bertolini's experiment (1910) on syphilitic and non syphilitic serum. Recently Lewis (1922) examined the ultraviolet absorption spectra of blood serum and stated that the absorption curve of pseudoglobulin is always constant and that of euglobulin differs from the former. The author (1920) distinguished the ultraviolet absorption spectra between the normal and the immune serum and also found (1922) that their absorptions in the same part of spectra are reversed by the antagonistic action of inorganic salts. Noüy (1922) reported on the surface equilibrium of blood serum and he gives many results on the restoration of the surface tension of serum, after a lowering caused by an addition of a surface acting substance. The author (1923) found the antagonistic action between salts on the surface tension of blood serum. Here we again undertook the following investigation on the dispersity and the surface tension of blood serum.

In the following experiments, we determined the dispersity of serum, comparing its ultraviolet absorption spectra by the use of a quartz spectroscope (I. D. 15 made by Adam Hilgard Co. in London). An electric spark of John's electrode and a hydrogen capillary tube were used as a source of light, discharging an alternating high voltage current obtained from a transformer. All photographs were taken through the same thickness of serum with the same ratio of light intensity using a photometer. Next

the surface tension of blood serum was determined in a constant temperature by the use of Noüy's apparatus (1919). The principle of this apparatus is based upon the fact that a metal ring adheres to the surface of the liquid and the torsion of the wire is used to counteract the tension of the liquid film and to break it; thus it is simply a torsion balance. As the force on the wire in case of water is only 75 dynes per sq. cm., we can assume that, within this limit the strain on the wire is proportional to the angle of torsion. All samples of serum used in my experiments were prepared from fresh blood and kept in an ice chest for few days. The samples of the surface tension experiment were treated 30 minutes at 58°C. and their surface tension was fixed, i. e. it is the so called "static surface tension".

I. The Dispersity of Blood Serum.

1. *Differences of ultraviolet-absorption spectra between rabbit-, dog- and horse-serum.*

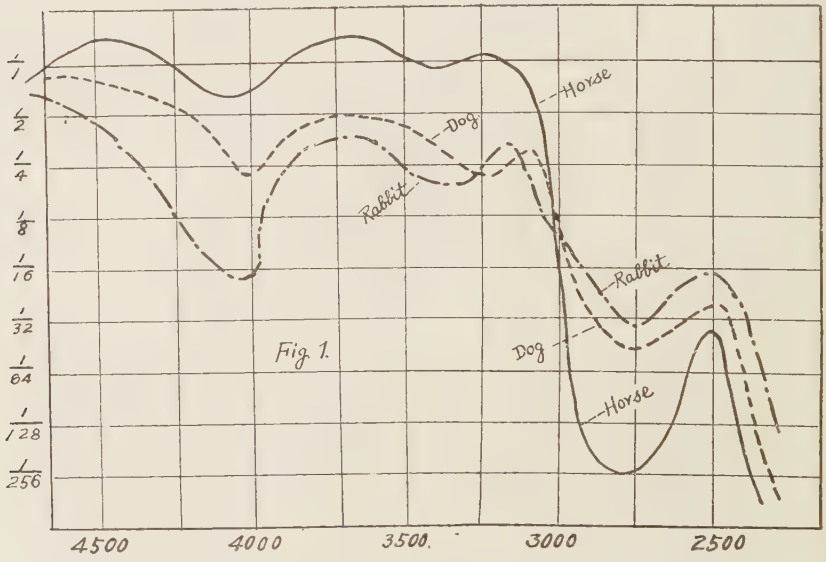
The blood serum preserved in an ice chamber was diluted with 0.85% NaCl solution, according to one of the following tables and photographed with Wratten and Wainwright "M" plate in 6 minutes exposure with a hydrogen tube (Tadokoro 1922). The results of the following table were the average numbers of the wave length of the boundary lines of the absorption bands in each 5–10 plates and the following differences were observed.

TABLE I.

Concentration of serum	The wave length of the boundary line of the absorption band		
	Horse serum	Dog serum	Rabbit serum
Original conc.	4750–4250–3825–3120	— 5800 — — —	— — —
1/2 Conc.	— — — 3070	— 5350–4905–4200 —	5800–5100–4500
1/4 Conc.	— — — 3030	4080–3952–3301–3105–3005	5740–5405–4320 –3905–3500–3100

Concentration of serum	The wave length of the boundary line of the absorption band		
	Horse serum	Dog serum	Rabbit serum
1/8 Conc.	— — — 3010	— — — — 3001	4250–3900–3090
1/16 Conc.	— — — 2990	— — — — 2990	4060–3950–2850
1/32 Conc.	— — — 2995	— — 2820–2625–2485	2770–2700–2490
1/64 Conc.	— 2990 2600–2494	— — — 2480 —	— — 2480
1/128 Conc.	— 2990–2650–2480	— — — 2480 —	— — 2470
1/256 Conc.	— 2860–2750–2480	— — — 2479 —	— — 2465

From the above results, each typical curve was traced in a coordinate system, taking the wave length in the abscissa and the concentration of serum in the ordinate and the following curves were obtained as shown in fig. 1.



The results of the above table and of the figure show the clear differences of ultraviolet absorption spectra between rabbit-,

dog- and horse-serum. This difference is not only the form of absorption curve but the absorption capacity in different concentration of serum, i. e. in part of λ 3000–2500 $\mu\mu$, the absorption band of rabbit serum becoming extinct in 1/32 dilution while that of dog serum remained unchanged and the absorption band of horse serum was observed until ca. 1/256 dilution of original serum.

2. *Change of ultraviolet-absorption spectra of human blood serum in menstruation.*

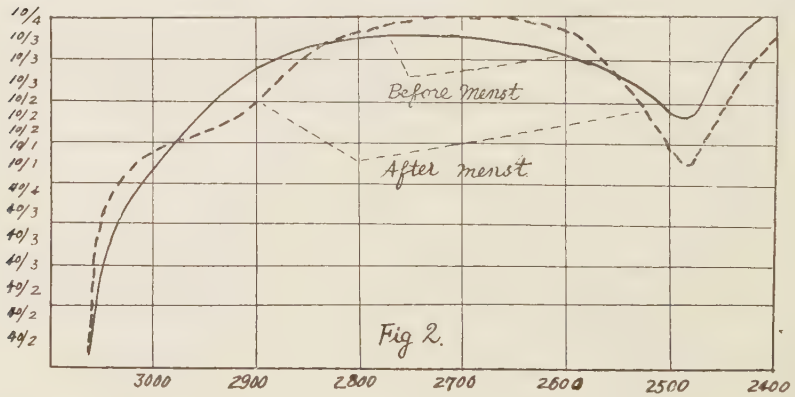
The blood serum preserved in an ice chamber was diluted to 1/100 with 0.85% NaCl solution and photographed with Shumann's special sensitive plate by the following different exposures and through the different thicknesses of liquid. The results of the following table are the average number of the wave lengths of the boundary line of the absorption band in the following samples, i. e. samples collected from different periods of the same 4 persons (before and after) 9 persons (before) and 4 persons (after), the total sum of samples corresponds to 13 (before) and 8 (after menstruation).

TABLE II.

Tickness of cell	Exposure minutes	The wave length of the boundary line of the absorption band	
		Before menstruation (the same result was obtained 1 week after menst.)	After menstruation (2–3 days after menst.)
10 mm	4	2445 — —	2380 — —
10 mm	3	2449 — —	2410–2560 —
10 mm	3	2450–2570–2880	2420–2560–2850
10 mm	3	2480–2550–2905	2440–2550–2870
10 mm	2	2472–2505–2945	2448–2532–2899
10 mm	2	— — 2965	2463–2519–2925
10 mm	2	— — 2981	2472–2502–2982
10 mm	1	— — 2995	2481–2492–3013
10 mm	1	— — 3012	— — 3311
40 mm	4	— — 3201	— — 3425

Tickness of cell	Exposure minutes	The wave length of the boundary line of the absorption band	
		Before menstruation (the same result was obtained 1 week after menst.)	After menstruation (2-3 days after menst.)
40 mm	3	— — 3350	— — 3501
40 mm	3	— — 3350	— — 3501
40 mm	3	— — 3411	— — 3558
40 mm	2	— — 3492	— — 3561
40 mm	2	— — 3556	— — 3565
40 mm	2	— — 3565	— — 3565
40 mm	1	— — 3565	— — 3565

From the above results, each typical curve was traced in a coordinate system, taking the wave length on the abscissa and the exposure in the ordinate and the following curves were obtained as shown in fig. 2.



The results of the above table and of the figure show the difference of the ultraviolet absorption spectra before and after menstruation. Immediately cessation of menstruation, the absorption band in λ 2600–2400 $\mu\mu$ part grows wide and deep and in λ 2850 $\mu\mu$ part becomes irregular. But after one week of cessation of mentruation, this change is no longer observed and the absorption band coincides with that of before menstruation.

II. The Surface Tension of Blood Serum.

3. *Difference of the surface tension between rat-, rabbit-, sheep-, horse- and human-serum.*

0.5 cc. of blood serum, preserved in an ice chamber (0–7.0°C) were taken on a dried watch glass and its surface tension was measured with Noüy's apparatus at 20°C. The following numbers express dynes per sq. cm. ($H_2O=75$ dynes/sq. cm.) and each result is an average of many samples.

TABLE III.

Name of serum	Numbers of experiments	Type A	Type B	Type C	Type D
Horse	From No. 1 to No. 8 (average)	—	61.8	62.5	—
Sheep	From No. 1 to No. 3 ,,	—	—	62.7	—
Rabbit	From No. 1 to No. 3 ,,	59.1	61.8	62.7	—
Rat	From No. 1 to No. 2 ,,	59.1	—	62.7	—
Human	From No. 1 to No. 28 ,,	59.1	61.8	62.7	57.2

From the above results, no difference was observed between the different kinds of animals, but there are many different types in the same kind of animal.

4. *Change of the surface tension of human serum in menstruation.*

Some samples were collected during three different period of menstruation (before, middle and after) from the same person and other samples from different persons.

TABLE IV.

Name of sample	The same person or not	Before menstruation	During menstruation	After menstruation	Long after menstruation
T. Z. I.	The same person	—	63.0	—	62.7
Y. B.	The same person	62.7	62.8	59.5	62.7
— (Average of 4)	Not the same person	62.7	—	—	—

The above results show the irregular change of surface tension during and after menstruation.

5. Difference of the surface tension of serum between woman and man.

TABLE V.

Woman or man. Number of person.	Type A	Type B	Type C	Type D
Man 18 persons	59.1	61.8	62.7	57.2
Woman 11 persons	59.1	61.8	62.7	—

From the above result, there is no difference between woman and man serum, though in both kinds of serum, the same different types were observed.

Resumé.

1) On the ultraviolet absorption spectra of blood serum, a great difference was observed in different kinds of animals and it is not only the form of absorption curve but also in its absorption capacity.

2) On the ultraviolet absorption spectra of woman serum, an irregular change was observed immediately after cessation of menstruation and after one week return to normal condition.

3) On the surface tension of blood serum, no difference was observed between the different kinds of animal, but there are different types of the same kind of animal.

4) On the surface tension of woman serum, an irregular change was observed midway and immediately after cessation of menstruation.

5) On the surface tension of blood serum, there was no difference between woman and man, but in both kinds of serum the same different type was observed.

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ÜBER DEN EINFLUSS DER EIWEISS-FETTDIÄT AUF DEN KOHLENHYDRATSTOFFWECHSEL.

IV. Mitteilung.

VON

NAOMI KAGEURA.

(*Aus der medizinischen Klinik von Prof. Dr. Ryokichi Inada, Kaiserliche Universität zu Tokyo*)

(Eingegangen am 21. Juli, 1923.)

In den vorangehenden Arbeiten habe ich mitgeteilt, dass kohlenhydratarme Diät, welche wesentlich aus Eiweiss und Fett besteht, die Assimilationskraft für Kohlenhydrat beim Menschen wie beim Hunde deutlich herabsetzt, was durch Kohlehydratzusatz zur Nahrung sofort beseitigt wird (I. Mitteil.). Dabei habe ich auch gefunden, dass Kohlenhydrat und Eiweiss resp. Fett gegeneinander in Bezug auf die Zuckerassimilation sozusagen antagonistisch wirken (II. Mitteil.). Nun soll gefragt werden, wie solche Erscheinungen zustande kommen. Die Azidosis scheint hier keine besondere Rolle zu spielen (III. Mitteil.). Die eigentliche Ursache dieser Erscheinungen muss also nach anderer Richtung hingesucht werden. In diese Frage tiefer einzudringen, habe ich auf Anraten und unter der Leitung von Herren Proff. R. Inada und K. Sakaguchi die Glykogenbildung in der Leber an kohlenhydratreich resp. -arm genährten Hunden vergleichend untersucht, indem ich dabei die Leber mit dem Blute von demselben Tiere unter Zusatz von reichlich Traubenzucker künstlich durchblutete.

Erste Versuchsreihe.

Sechs kohlenhydratreich genährte Hunde, deren Assimilationskraft für Zucker vorher bei Untersuchung alimentärer Hyper-

glykämie (nach Verabreichung von 50 g Traubenzucker in 200 ccm Wasser) sich normal zeigte, wurden 20 Stunden nach der letzten Nahrungsaufnahme unter Morphiumnarkose möglichst weitgehend entblutet. Dann wurde die Leber vollends rasch herausgenommen und gewogen. Ein Läppchen davon (22-46 g) diente als Kontrolle zu direkter Bestimmung des Glykogengehaltes. Der übrige Hauptanteil der Leber wurde an den Durchblutungsapparat von Yamakawa mit Mizukis Pumpe angeschlossen. Durch sie wurde das von demselben Tiere gewonnene defibrinierte Blut getrieben, welches mit gleichem Teile von einer Flüssigkeit verdünnt war, die nach Vorschlag Barrenscheens aus $\frac{4}{5}$ Teilen einer Lösung (NaCl 0.9%, $CaCl_2$ 0.024%, KCl 0.042%, $NaHCO_3$ 0.03%, Traubenzucker 0.08) und $\frac{1}{5}$ Teil von 20% Traubenzuckerlösung bestand. Nach einstündiger Durchblutung wurde der Glykogengehalt der Leber wieder mit Mori-Iwasakis Methode bestimmt. Die einzelnen Daten sind in einer Tabelle übersichtlich zusammengestellt.

TABELLE I.

Nr. d. Hundes		Gewicht* d. Leber (g)	Glykogengehalt (%)	Glykogengehalt (g)	Zunahme (g)	Zunahme (%)
1	vor d. Durchblut.	524	0.52	2.72		
	nach „ „	900	0.67 (1.15)**	6.03	3.31	122
2	vor „ „	338	0.72	2.43		
	nach „ „	510	0.94 (1.42)	4.79	2.36	97
3	vor „ „	395	0.70	2.77		
	nach „ „	625	1.04 (1.65)	6.50	3.73	133

Nr. d. Hundes		Gewicht* d. Leber (g)	Glykogengehalt (%)	Glykogengehalt (g)	Zunahme (g)	Zunahme (%)
4	vor „ „	479	0.67	3.21		
	nach „ „	800	1.17 (1.95)	9.36	6.15	192
5	vor „ „	574	0.42	2.43		
	nach „ „	860	0.57 (0.88)	4.90	2.57	110
6	vor „ „	702	0.72	5.05		
	nach „ „	1160	0.94 (1.55)	10.90	5.85	116

** Die eingeklammerte Zahl zeigt den auf das Lebergewicht vor der Durchblutung umgerechneten Wert.

* Hier ist nicht das totale Lebergewicht, sondern nur das Gewicht des zur Durchblutung genommenen Leberanteils angegeben.

Die Tabelle zeigt, dass das Leberglykogen bei kohlenhydratreich genährten Hunden bei Durchblutungsversuchen, welche unter den oben geschilderten Bedingungen ausgeführt wurden, deutlich zunimmt. Bei meinen Untersuchungen an sechs Hunden war der Glykogengehalt der Leber vor der Durchblutung 0.42–0.72 Proz. und danach 0.88–1.95 Proz. Seine Zunahme betrug 97–192 Proz.

Zweite Versuchsreihe.

An sechs Hunden wurde zuerst durch Untersuchung der alimentären Hyperglykämie bestätigt, dass zweitägige Eiweiss-Fettdiät sicher eine deutliche Herabsetzung der Assimilationsfähigkeit für Kohlenhydrat nach sich zieht. Hierauf habe ich die Tiere wieder zwei Tage lang mit derselben Kost gefüttert. Nun wurden sie nach 20-stündigem Fasten getötet und die Durchblutung ganz in gleicher Weise wie bei vorangehenden Versuchen ausgeführt.

TABELLE II.

Nr. d. Hundes		Gewicht* d. Leber (g)	Glykogengehalt (%)	Glykogengehalt (g)	Zunahme (g)	Zunahme (%)
7	vor d. Durchblut.	380	0.52	1.98	0.33	17
	nach „ „	625	0.37 (0.61)**	2.31		
8	vor „ „	270	0.55	1.49	1.01	68
	nach „ „	480	0.52 (0.92)	2.50		
9	vor „ „	256	0.67	1.72	1.03	60
	nach „ „	417	0.66 (1.09)	2.75		
10	vor „ „	407	0.47	1.91	0.43	23
	nach „ „	650	0.36 (0.57)	2.34		
11	vor „ „	434	0.54	2.34	0.61	26
	nach „ „	670	0.44 (0.68)	2.95		
12	vor „ „	276	0.46	1.27	0.78	61
	nach „ „	500	0.41 (0.75)	2.05		

* ** Die Fussnoten in der Tabelle I. gelten auch für hier.

Aus der Tabelle ersieht man, dass die Zunahme des Leberglykogens nach der Durchblutung bei kohlenhydratarm genährten Hunden relativ unbedeutend ist. Vor der Durchblutung betrug der Glykogengehalt der Leber 0.46–0.67 Proz. und nach der Durchblutung 0.61–1.09 Proz. Die relative Zunahme beträgt hier nur 17–67 Proz.

Zusammenfassung.

1. Wenn das Tier nach 20-stündigem Fasten getötet wird, so zeigt der Glykogengehalt der Leber bei kohlenhydratreich wie -arm genährten Hunden einen fast gleichen Wert, nämlich bei jenen 0.42–0.72 Proz. und bei diesen 0.46–0.67 Proz.

2. Bei Durchblutungsversuchen wurde der Glykogenansatz der Leber bei kohlenhydratreich genährten Hunden sehr auffallend (97–192 Proz.), aber bei den mit Eiweiss-Fettdiät gefütterten nur unbedeutend (17–67 Proz.) gefunden.

3. Diese Herabsetzung der Glykogenbildung in der Leber soll für die Beeinträchtigung der Zuckerassimilation durch Eiweiss-Fettdiät sicher ein ätiologisches Moment darstellen.

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STUDIES ON CATALYTIC AND OXIDATIVE ACTIVITY.

By

TAIZO KIMURA.

(*From the Biochemical Laboratory, Institute of Medical Chemistry, Tokyo Imperial University. Director: Prof. S. Kakiuchi.*)

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It is often reported that there is always a trace of metals, such as iron, magnesium or others, contained in enzymes which act catalytically or oxidatively. The significance of the presence of metal is implicitly recognized by many authors. The mode of action is, however, still not clear. Moreover, the relation between catalas and peroxidase in the oxidative activity of cells has to be examined.

In view of getting some insight in this matter, the present work was undertaken and its result is given in the following pages.

I. Material and Method.

A. Material.

1. Hydrogen peroxide.

Hydrogen peroxide used for my experiments was partly the pre-war samples of Merck and partly obtained from Sankyo. The preparation obtained from Merck after the war was found too impure to be used and it was only through the kindness of Sankyo Co., that I was able to prepare a pure sample of it without any contamination of impurities. Just previous to its use, it was brought to the acidity pH=5 by adding N/100 NaOH using Clark's indicator method and diluted with distilled water to the wanted concentration.

2. Test-tube and flask.

A number of test-tubes of ordinary soft glass, Nakano's and Jena's were steeped in crude concentrated hydrochloric acid for two days, washed with tap water and finally rinsed with distilled water. To each of them were added 5 cc. of 0.05% fuchsine solution and 5 drops of 20% hydrogen peroxide (Merck).

The mixture was then boiled on the flame of Bunsen's burner for a minute and left overnight. In test-tubes of soft glass the colour became faint markedly, while in Jena's and Nakano's glasses only a little fading of colour was observed. Even when the glasses were steeped in crude concentrated hydrochloric acid and nitric acid successively, a week for each, or boiled in the same solution two days for each, the fading of colour did not improve much. It must be, therefore, concluded that this fading away of colour is not much due to the impurity of the glass and is entirely owing to the action of hydrogen peroxide upon fuchsine. This relation has been studied lately by Karczag (1922).

In all of the following experiments, I have used glass ware of all sorts made of Nakano's hard glass after treating them with hydrochloric acid for two days.

B. Method.

1. The determination of hydrogen peroxide and its decomposition.

The hydrogen peroxide which remained after its decomposition was determined either by means of titration or by volumetric estimation in the presence of sulphuric acid with 1/10 normal potassium permanganate solution. If there is, however, any organic matter as fuchsine, alcohol, or albumin etc., present, it may give us an incorrect value, and it becomes necessary to correct it with a blind test by letting potassium permanganate act on the same amount of organic substances in question.

The volume of oxygen which was evolved from hydrogen

peroxide by the catalase activity was measured volumetrically.

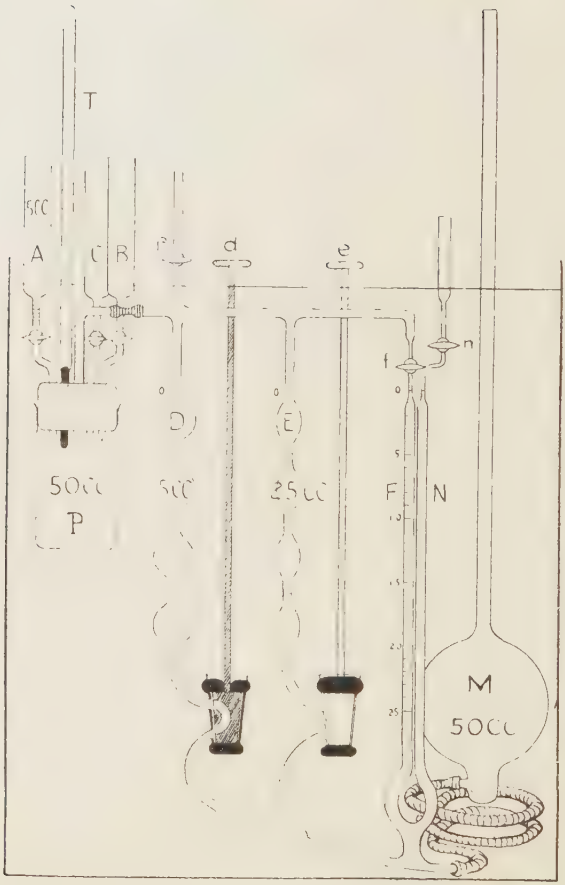
An apparatus was devised which enabled me to measure a minute amount of oxygen produced by the decomposition of hydrogen peroxide. To avoid any error caused by the chance moment the control test was always performed, in which the same amount of water was used instead of the reagent considered.

2. Apparatus for gas-volumetric determination.

a. *The apparatus.* The apparatus used in my experiments for the determination of gas volume is the one modified from Gaffcken's apparatus and is shown in the figure. It consists of 50 cc. decomposition flask (P), three 5 cc. burettes (A. B. C.), a thermometer (T), three measuring tubes for 20 cc. (D) 10 cc. (E) 2.5 cc. (F) each, a levelling tube (N) and the mercury reservoir (M). D and E have four bulbs, each of exactly 5 cc. or 2.5 cc. volume, and mercury in them can be raised or drained off by raising or lowering the reservoir M after the connection is made between M by means of the cock *d* or *e*: all are connected together as shown in the figure.

b. *The method of determining the gas volume.* At first all cocks are opened, and the air in the measuring tube is driven off by raising the reservoir M until the mercury rises in the capillary up to the cocks *d*, *e*, *f* and *n*. Then the cocks are closed, M is lowered and the air when occulted in the mercury and adhering on the wall of the tubes and India rubber tube, accumulates at the top of each tube. This is driven out again as before. The procedure is repeated until no more air appears.

The reagents are measured into the flask P and the whole apparatus is placed in a thermostat at 30°C. When the temperature attained 30°C, the cocks *d*, *e*, *f* and *n* are opened and M is raised until the mercury rises in the capillary of the measuring tubes D, E and F up to the mark O. Then the cocks *d*, *e* and *r* are closed and after 5 minutes M is adjusted so as the mercury of the tubes F and N are levelled, and its height in F is read



(V′). Then the cocks *d* and *e* are opened, M is lowered and the reagents in the burettes (A. B.) are allowed to run into the flask P. After a definite interval, the reaction is checked by addition of a necessary amount of sulphuric acid from the burette C and the flask P is shaken slowly for 5 minutes. The height of the mercury in D and E is suitably adjusted by controlling M according to the volume of the reagent added and the gas evolved and cocks *d* and *e* are closed. M is raised or lowered

until the mercury of the tubes F and N stands at the same level and its position in the tube F is read (V). Then the difference V-V′ is the gas which is evolved. The volume of the measured gas is reduced to 0°C and 760 mm. pressure.

II. Catalytic and Oxidative Activity of Heavy Metal Salts.

A. Catalytic activity of heavy metal salts.

It has been worked out by Bohnson (1912) and Duclaux (1922) that the decomposition of hydrogen peroxide by iron salt belongs to the mono-molecular reaction and the velocity of reaction is proportional to the concentration of iron ion, and that the ferric and ferrous ion are both active. In the following experiments I have examined many factors regarding the catalytic activity of metallic ion on hydrogen peroxide.

Experiment 1. Catalytic decomposition of hydrogen peroxide by salt of heavy metals.

To 4 cc. of 0.75% H_2O_2 (pH=5) in a number of Erlenmeyer flasks was added 1 cc. of varying concentration of freshly prepared solution, such as $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $HgCl_2$, $SbCl_2$, $ZnCl_2$, $Pb(NO_3)_2$, $AuCl_3$, and $PtCl_4$, and the flasks were placed in a thermostat at 30°C. At the definite intervals, as mentioned below, the reaction was checked by the addition of 5 cc. of H_2SO_4 (1 : 4). 2 cc. of the mixture were pipetted out into an Erlenmeyer flask, diluted with distilled water and the amount of H_2O_2 was titrated with N/10 $KMnO_4$. The result is shown in the following table.

TABLE I.

Iron chloride.

Conc. of $FeCl_3$	Amount of H_2O_2 present (expressed in terms of cc of N/10 $KMnO_4$ used) after the lapse of							
	15m.	30m.	45m.	1h.	1.5h.	2h.	2.5h.	3h.
0	3.66							3.66
M/6400		3.59		3.59		3.59		3.58
M/3200		3.54		3.51		3.45		3.4
M/1600		3.3		3.2		2.98		2.81
M/800	2.57	2.18	2.00	1.83	1.63	1.48	1.33	1.23
M/400	1.6	0.85	0.59	0.425	0.275	0.155	0.105	0.08
M/200	0.545	0.16	0.075	0.05				
M/100	0.235	0.07	0.03					
M/50	0.145	0.05	0.03					

TABLE II.

Copper chloride.

Conc. of $CuCl_2$	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of					
	30m.	1h.	1.5h.	2h.	2.5h.	3h.
0	3.56					3.56
M/6400			3.5			3.44
M/3200			3.4			3.33
M/1600			3.1			2.73
M/800	3.2	2.82		2.34		2.18
M/400	3.0	2.45		1.9		1.65
M/200	2.75	2.0	1.64	1.375		1.0
M/100	1.9	1.32	0.9	0.65	0.47	0.37
M/50	1.9	1.1	0.57	0.29	0.16	0.11

TABLE III.

Salt of other heavy metals.

Kind of salt sol.	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
0	3.65			3.65
M/50 $CoCl_2$	3.54	3.23	3.04	2.72
M/50 $NiCl_2$	3.57	3.52	3.52	3.51
M/50 $Pb(NO_3)_2$	3.6	3.6	3.6	3.6
M/50 $ZnCl_2$	3.58	3.58	3.58	3.56
M/50 $HgCl_2$	3.58	3.56	3.56	3.56
M/100 $AuCl_3$	3.59	3.26	3.26	3.26
M/50 $AuCl_3$		3.43	3.43	3.43
M/50 $PtCl_4$	3.52	3.22	3.02	2.92
M/50 $SbCl_2$	3.45	3.45	3.45	3.44

As the table shows, iron chloride is the most active, copper chloride the second, and the others are all feeble in activity under such conditions as the above.

It is also quite noticeable that while the iron chloride, more concentrated than 1/800 mol, is stronger in its activity than the copper salt solution of the same concentration, it is much weaker than the copper salt solution in the case of the lower concentration. This is probably due to the stronger hydrolysis of iron salt in these weak concentrations than those of copper salt.

Experiment 2. The comparison of activity of freshly prepared and old iron chloride solution.

It seems to be conceivable from the fact of the above experiments that the activity of iron salt solution must decrease during the course of many days of preservation owing to the

increase of hydrolysis. That this is really the case will be seen from the following experiment.

To 4 cc. of 0.75% H_2O_2 1 cc. of freshly prepared or old M/200 $FeCl_3$ solution was added and kept in a thermostat at 30°C. After the lapse of 1, 2 and 3 hours 5 cc. of H_2SO_4 (1 : 4) were added to check the reaction. 2 cc. of the mixture were then pipetted out, diluted with distilled water, and the amout of H_2O_2 was titrated with N/10 $KMnO_4$.

The result is given in the following table.

TABLE IV.

Age of $FeCl_3$ solution	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
day fresh	3.62	2.85	2.5	2.24
2		2.99	2.62	2.3
3		3.05	2.75	2.5
4		3.16	2.89	2.7
5		3.16	2.91	2.72
6		3.28	3.03	2.81
7		3.32	3.06	2.89
8		3.46	3.4	3.18
9		3.51	3.43	3.38

Experiment 3. The influence of hydrogen ion concentration.

The influence of the reaction of the system also reveals to us that the concentration of metallic ion is the important factor of the activity. This is shown in the following experiments.

0.75% hydrogen peroxide solution with the definite value pH=3, 4, 5, 6, 7, 8, 9 and 10 were prepared from hydrochloric acid and sodium hydroxide controlled by Clark's indicator method. To 4 cc. of each of such solutions 1 cc. of $FeCl_3$ solution of varying concentrations was added. At the definite intervals, as mentioned below, the reaction was checked by the addition of

5 cc. of H_2SO_4 (1 : 4). 2 cc. of the mixture were then pipetted out into an Erlenmeyer flask, diluted with distilled water, and the amount of H_2O_2 was titrated with N/10 $KMnO_4$.

The results are given in the following tables.

TABLE V.

Endconc. of $FeCl_3$ = M/400. Room temp. 31°C.
Initial conc. of H_2O_2 corresponds to 3.65 cc. of N/10 $KMnO_4$ used.

pH value of H_2O_2 added	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of	
	30m.	1h.
3	0.55	0.2
4	0.98	0.49
5	1.02	0.52
6	1.05	0.55
7	1.23	0.68
8	1.24	0.69
9	1.25	0.71
10	1.29	0.74

TABLE VI.

Endconc. of $FeCl_3$ M/800. Room temp. 31°C.
Initial conc. of H_2O_2 corresponds to 3.65 cc. of N/10 $KMnO_4$ used.

pH value of H_2O_2 added	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of		
	1h.	2h.	3h.
3	0.88	0.45	0.25
4	1.96	1.47	1.14
5	2.00	1.59	1.24
6	2.20	1.83	1.52
7	2.30	2.15	1.83
8	2.30	2.15	1.83
9	2.46	2.24	1.95
10	2.50	2.27	1.99

The same experiment was performed with copper chloride.

TABLE VII.

Endconc. of $CuCl_2$ M/400. Room temp. 30°C.
Initial concentration of H_2O_2 corresponds to 3.65 cc. of N/10 $KMnO_4$ used.

pH value of H_2O_2 added	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of		
	1h.	2h.	3h.
4	2.5	2.08	1.74
5	2.5	2.08	1.76
6	2.53	2.08	1.78
7	2.56	2.14	1.83
8	2.56	2.17	1.84
9	2.56	2.19	1.88
10	2.61	2.26	1.95

B. Oxidative activity of heavy metal salts.

Karczag (1921) studied the decolorizing effect of hydrogen peroxide on the dilute solution of many organic pigments in the presence of salt of heavy metal, and came to the conclusion that iron acts as the "cold oxidizer," while copper and manganese belong to the "heat-oxidizers." The former can decolorize the dyes in the cold state, whereas copper acts more slowly in the cold, but acts best when heat is applied. He classified nickel and platinum, as the third group, in which the decolorizing activity is quite elective.

He also stated that "cold and heat oxidizers" possess in the system pigment + oxidizers + H_2O_2 the activity of oxidase catalase types.

As it seemed to me quite interesting to follow the relation between the oxidative and catalytic activities quantitatively, I have made the following experiments.

Experiment 1. Influence of oligodynamic action.

It has been reported by Fatta and Richter-Quittner

(1921) that the beaker in which the metal as Cu, Hg, Ag, Pb, Sn, Zn, Al, Fe, Mg or Pt was placed with water for eight days, acquired even after rinsing it with distilled water, the property of oxiding many organic substances. In fear of being disturbed in the course of my study by such an oligodynamic action in the container of the salt of heavy metals even after rinsing, the following test was conducted.

Each of a number of purified test-tubes of the same calibre was supplied with 5 cc. of 1% $FeCl_3$ solution and left for a varying lapse of time from 30 minutes to 7 days. The tube was then emptied and washed five times with distilled water. To each of the test-tubes thus treated, 5 cc. of 0.05 % fuchsine solution and 5 drops of 20% H_2O_2 were added. The mixture was then boiled on the flame of Bunsen's burner, left at the room temperature and the change of the colour tone was observed for a day. All the test-tubes showed the same colour tone as that which was not treated with iron chloride.

In the experiment, in which nickel chloride or cobalt chloride was used instead of iron chloride, the results were just the same as the case mentioned above.

It is therefore safe to assume that in the method used in my study there is no fear of disturbances caused by this moment.

Experiment 2. Oxidative activity of salt of heavy metals.

To each of the purified test-tubes were added 4 cc. of 0.05% fuchsine, 4 drops of 20% H_2O_2 (pH=5) and 1 cc. of varying concentration of salt solutions of heavy metal, such as $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $Pb(NO_3)_2$, $HgCl_2$, $MnCl_2$, $AuCl_3$, or $PtCl_4$. The mixture was then boiled on the flame of Bunsen's burner for a minute and the change of the colour tone was observed directly and also after 1 hour. The results are given in the following tables.

TABLE VIII.

Iron chloride.

Conc. of $FeCl_3$ solution	Colour of $FeCl_3$ solution	Change of colour				
		After the addition of fuchsine	After the addition of H_2O_2	On boiling	After cooling	
					Solution	Precipitate
M/50	light yellow	dark red	foams a little and the colour becomes a little deeper	Black precipitate is formed at once, which under active foaming dissolves quite soon to a clear brown solution.	brown	brown
M/100	colour gradually decreases	colour gradually decreases			colour gradually decreases	amount of precipitate gradually decreases
M/200			,,			
M/400			,,	The reaction is gradual and tardy.		
M/800			,,	dark turbid red		
M/1600	colourless	same as control	same as control	,,	trace of yellow	no precipitation
M/3200				,,	dark turbid red	
M/6400				deep red, no foam	,,	
M/12800				,,	,,	
M/25600				red	red	
M/51200				same as control	same as control	
0 (control)				a little faint	a little faint	

TABLE IX.

Copper chloride.

Conc. of $CuCl_2$ solution	Colour of $CuCl_2$ solution	Chage of colour: After the addition of fuchsine	Chage of colour: After the addition of H_2O_2	Chage of colour: On boiling	Chage of colour: After cooling: Solution	Chage of colour: After cooling: Precipitate
M/50	light blue	deeper	changeless	Black precipitate is formed at once, which under active foaming dissolves quite soon to a clear solution.	trace of blue	brown
M/100	colour gradually decreases	,,			,,	decreases, ⟶
M/200		,,			,,	
M/400	colourless	same as control			,,	
M/800				The reaction is gradual and tardy.	trace of purple	amount of precipitate gradually increases, ⟶
M/1600					light purple	
M/3200				,,	,,	
M/6400				dark red	dark red turbid	decreases, ⟶
M/12800				deep red	,,	
M/25600				,,	,,	
M/51200				same as control	clear red	no precipitation
M/102400				,,	same as control	
0 (control)				a little faint	a little faint	

TABLE X.

Gold chloride.

Conc. of $AuCl_3$ solution	Colour of $AuCl_3$ solution	Change of colour				
		After the addition of fuchsine	After the addition of H_2O_2	On boiling	After cooling	
					Solution	Precipitate
M/50	yellow	light yellow with grey precipitate	foams a little	light purple with grey precipitate	trace of purple	grey
M/100	colour gradually decreases	colour and precipitate gradually decrease		"	"	"
M/200				"	"	"
M/400				"	trace of rose	"
M/800		dark turbid red		"	"	"
M/1600		turbility gradually decreases		dark turbid red	dark turbid red	no precipitation
M/3200				"	deep red	
M/6400	colourless			deep red	colour gradually decreases	
M/12800		dark red		"		
M/25600		red		red		
M/51200		same as control		same as control	same as control	
M/102400		"		"	"	
0 (control)				a little faint	a little faint	

TABLE XI.

Platinic chloride

Conc. of $PtCl_4$ solution	Colour of $PtCl_4$ solution	Change of colour				
		After the addition of fuchsine	After the addition of H_2O_2	On boiling	After cooling	
					Solution	Precipitate
M/50	yellow	pink turbid	foams a little	foams a little	light yellow	grey
M/100	colour gradually decreases	turbidity gradually decreases			"	"
M/200					"	brownish
M/400					trace of purple	"
M/800		trace of turbidity			trace of rose	"
M/1600		"			"	no precipitation
M/3200	trace of yellow	same as control			redish turbid	
M/6400					colour gradually decreases	
M/12800						
M/25600						
M/51200					same as control	
M/102400					"	
0 (control)					a little faint	

TABLE XII.

Cobalt chloride.

Conc. of $CoCl_2$ solution	Colour of $CoCl_2$ solution	Change of colour				
		After the addition of fuchsine	After the addition of H_2O_2	On boiling	After cooling	
					Solution	Precipitate
M/50	light pink	deep red	changeless	a few foams	a clear red	no precipitation
M/100	colour gradually decreases	,,			colour gradually decreases	
M/200		,,				
M/400		same as control				
M/800						
M/1600	colourless					
M/3200					same as control	
0 (control)					a little faint	

TABLE XIII.

Nickel chloride.

Conc. of $NiCl_2$ solution	Colour of $NiCl_2$ solution	Change of colour				
		After the addition of fuchsine	After the addition of H_2O_2	On boiling	After cooling	
					Solution	Precipitate
M/50	light blue	deep red	changeless	a few foams	clear red	no precipitation
M/100	,,	,,			colour gradually decreases	
M/200	,,	,,				
M/400	colourless	same as control				
M/800						
M/1600					same as control	
0 (control)					a little faint	

TABLE XIV.

Salt of other heavy metals.

Kind and conc. of salt solution	Colour of salt solution	Change of colour				
		After the addition of fuchsine	After the addition of H_2O_2	On boiling	After cooling	
					Solution	Precipitate
$Pb(NO_3)_2$						
M/50	colourless	slight turbidity	changeless	a little faint	slight turbidity	no precipitation
M/100		"			"	
M/200		same as control			same as control	
$ZnCl_2$						
M/50	colourless	deep red	changeless	a little faint	orange yellow	a little
M/100		same as control			"	"
M/200		"			"	"
M/400		"			same as control	
$HgCl_2$						
M/50	colourless	slight turbidity	changeless	a little faint	turbid red	no precipitation
M/100		"			"	
M/200		trace			"	
M/400		same as control			same as control	
$MnCl_2$						
M/50	light pink	deep red	changeless	a little faint	a little faint	no precipitation
M/100	"	same as control			same as control	
M/200	"	"			"	
M/400	colourless	"			"	
0 (control)			changeless	a little faint	a little faint	

As the tables illustrated above, iron chloride acts strongly even at room temperature (10°C), and the change of colour is most distinct. At room temperature the colour gradually becomes faint and discolourized in the course of several hours to days differing according to the concentration of iron chloride and temperature. Copper chloride showed almost no change of colour at room temperature, while its reaction becomes quite active when heated. Gold chloride and platinic chloride produce a precipitation with fuchsine, and their reactions are not distinct. The action of cobalt chloride, nickel chloride, and all the others are also very weak.

Experiment 3. Study of fuchsine treated with hydrogen peroxide and iron chloride.

As experiment 2 shows, when the mixture of fuchsine, hydrogen peroxide and iron chloride at a definite proportion is boiled, black precipitate separates out, which under foaming soon dissolves in a colourless transparent solution. With a view to getting some idea of the nature of this precipitate, the reaction was interrupted by cooling as soon as the precipitate has appeared. The solution was then decanted off and centrifuged. The black mass thus obtained was washed with distilled water until the disappearance of the reaction of iron ion in the wash water and then dried by alcohol and ether, placed in a desiccator over sulphuric acid and paraffin, the mass becoming lustrous (a). When the concentration of iron chloride in the mixture was lower (b) or the quantity of hydrogen peroxide was less (c) the black precipitate was also obtained, which however did not dissolve even when heated. Treating these precipitates in the same way as the previous one, they were obtained in a pure condition.

The quantity of nitrogen and iron contained in these three precipitates was estimated as follows. In this experiment nitrogen was determined by Kjeldahl's method and iron by Zimmermann and Rheinhardt's.

TABLE XV.

Precipitate	Obtained from the mixture			N	Fe
	0.05% Fuchsine	$FeCl_3$	20% H_2O_2		
A	400 cc.	M/40 100 cc.	30 cc.	5.576%	7.62%
B	400 cc.	M/80 100 cc.	30 cc.	6.613%	3.259%
C	400 cc.	M/40 100 cc.	10 cc.	6.164%	2.232%
Fuchsine				10.438%	0

From this result it is evident that fuchsine or rather its decomposition product in a certain stage forms a compound combining with iron, the content of the latter being variable according to the stage of oxidation.

Experiment 4. Catalytic and oxidative activity played in the system of iron salt + fuchsine + H_2O_2.

As can be seen from the above experiment, the precipitate in the mixture of iron chloride, fuchsine and hydrogen peroxide contains iron in an insoluble form, so that the concentration of iron in the solution decreases. It follows therefore that the catalytic activity of iron salt must be weakened accordingly. To study the influence of dyestuff on the catalytic activity and to find out the amount of hydrogen peroxide necessary to the oxidation of dyestuff the following experiments were conducted.

0.8 cc. of 0.75% H_2O_2 containing 0.05% fuchsine was measured into a flask of the author's volumetric apparatus, kept in a water bath at 30°C. and 0.2 cc. of $FeCl_3$ solution of varying concentration was added. At the definite interval 5 cc. of H_2SO_4 (1 : 8) were then added to check the reaction. After shaking the mixture slowly for five minutes the oxygen evolved was measured (a). 5 cc. of N/10 $KMnO_4$ were then added, shaken again and the amount of H_2O_2 was estimated (b).

For the comparative study the same experiment without fuchsine was performed.

The result of these experiments is shown in the following tables.

TABLE XVI.

The amount of H_2O_2 (mg.) evolved by catalytic action of iron and used for the oxidation of fuchsine.

Endconc. of $FeCl_3$ M/50 :

Time		a Catalytically decomposed	b Rest in the remaining solution	a+b	Difference of (a+b) between control and fuchsine test	Colour	Precipitate
		mg.	*mg.*	*mg.*	*mg.*		
5′	Control	4.370	2.300	6.670	0.235	brown	brown
	Fuchsine	2.875	3.560	6.435			
15′	Control	6.161	0.400	6.561	0.729	faint	—
	Fuchsine	5.065	0.767	5.832			
30′	Control	6.708	0.137	6.845	0.734	faint	—
	Fuchsine	5.837	0.274	6.111			

Endconc. of $FeCl_3$ M/200 ;

15′	Control	4.792	1.820	6.612	0.297	reddish brown	brown
	Fuchsine	3.166	3.149	6.315			
30′	Control	6.078	0.424	6.502	0.451	brownish discolored	a little
	Fuchsine	4.545	1.506	6.051			
1.5h.	Control	6.708	0.137	6.845	0.758	faint	—
	Fuchsine	5.813	0.274	6.087			

TABLE XVII.

Endconc. of $FeCl_3$ M/400 :

Time		a Catalytically decomposed	b Rest in the remaining solution	a+b	Difference of (a+b) between control and fuchsine test	Colour	Precipitate
1 h.	Control	4.764	2.259	7.023	0.177	reddish	brown
	Fuchsine	3.149	3.697	6.846			
2 h.	Control	6.160	0.821	6.981	0.721	brownish discoloured	trace
	Fuchsine	3.422	2.838	6.26			
3 h.	Control	6.708	0.370	7.078	0.782	very faint, almost gone	trace
	Fuchsine	5.338	0.958	6.296			

Endconc. of $FeCl_3$ M/800 ;

1 h.	Control	2.793	4.162	6.955	0.11	reddish	brown
	Fuchsine	0.958	5.887	6.845			
2 h.	Control	3.642	3.327	6.969	0.134	reddish discoloured	brown
	Fuchsine	1.506	5.329	6.835			
3 h.	Control	4.655	2.327	6.982	0.616	brownish discoloured	trace
	Fuchsine	1.917	4.449	6.366			

From the tables we can deduce the following conclusion.

1. The value (a) is always smaller in the solution containing fuchsine, while (b) is larger in it than that of the solution free from fuchsine. This naturally indicates that the catalase activity of iron chloride was diminished by the presence of fuchsine.

2. The value (a+b) of the solution containing fuchsine is always smaller than that of the solution free from fuchsine. Its difference is due to the quantity of hydrogen peroxide used in the oxydation of fuchsine.

III. Influence of Caolin on the Activity of Iron Chloride on Hydrogen Peroxide.

As we have seen in the above experiments, the catalytic activity of iron salt depends entirely on the concentration of iron ion. What would result if we put some adsorbing material into the system? The decrease of iron ion in the solution would retard the reaction, while the concentration on the active surface would favour the decomposition of hydrogen peroxide. To see what sort of relation here exists the following experiments were undertaken.

Experiment 1. To 1 cc. of M/160 $FeCl_3$ in a number of Erlenmeyer flasks a varying amount of caolin and 5 cc. of 0.2% H_2O_2 were added and after the lapse of definite intervals, the reaction was inhibited by the addition of 5 cc. of H_2SO_4 (1 : 4). The amount of H_2O_2 was estimated by titration with N/10 $KMnO_4$, the result of which is given in the following table.

TABLE XVIII.

Quantity of caolin added	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of					
	0	1h.	2h.	3h.	4h.	5h.
gm. 0	5.8	3.24	2.73	2.2	1.9	1.66
0.01		2.66	1.67	1.25	0.8	0.63
0.05		3.48	2.67	2.07	1.63	1.26
0.1		4.19	3.15	2.5	2.00	1.74
0.25		4.78	4.25	3.32	2.85	2.7

As the table shows, the caolin at the definite concentration promotes the decomposition of hydrogen peroxide by iron chloride, while the excess of caolin diminishes the activity of iron salt.

Experiment 2. That a certain amount of caolin has a promoting effect for the catalytic activity of iron on hydrogen peroxide seems to lie in the fact that it gives a favorable surface for the process. Madinaveita and Aguirrechs (1921) pointed

out that the micellar magnitude has the deciding power on the catalytic activity.

We then tried to find out what result would be obtained when caolin was used which had been previously treated with iron chloride solution at different times. The treatment with iron chloride might change the nature of the surface.

To 0.5 gm. of caolin in each of the centrifuge tubes, 10 cc. of M/160 $FeCl_3$ were added, and the mixture was shaken thoroughly. After the lapse of 24 hours, the mixtures were centrifuged, the supernatant solution decanted off, and in most of the cases the residue was treated again with a fresh $FeCl_3$ solution, centrifuged after 24 hours, and so on. Several tubes containing the caolin treated with $FeCl_3$ from 1 to 6 times were prepared by the repetition of this procedure and to each of such tubes was added 1 cc. of M/160 $FeCl_3$ and 5 cc. of 0.75% H_2O_2. After the lapse of 1, 2 and 3 hours, the reaction was checked by the addition of 6 cc. of H_2SO_4 (1 : 4). 2 cc. of the solution were pipetted out, diluted with distilled water and the amount of H_2O_2 was estimated by titration with N/10 $KMnO_4$.

The result is shown in the following table.

TABLE XIX.

Room temp. 31°C.

Number of treatment of iron caolin	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
0	3.5			
1		1.92	1.16	0.63
2		1.97	1.3	0.76
3		2.06	1.4	0.85
4		2·1	1.41	0.88
5		2.1	1.43	0.92
6		2.2	1.55	1.03

Experiment 3. The above experiment was repeated again with modification that the caolin treated with iron chloride was washed with distilled water completely until the reaction of iron ion entirely disappeared from the wash water before it was treated with a fresh iron chloride solution.

On the other hand, the same procedure was conducted in the presence of fuchsine at the rate of 0.04%. The results are shown in the following table.

TABLE XX.

Room temp. 30°C.

Number of treatment of iron caolin	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of							
	Without fuchsine				With fuchsine			
	0	1h.	2h.	3h.	0	1h.	2h.	3h.
0	3.5			3.5	3.5			3.5
1		2.78	1.98	1.39		2.7	1.5	0.46
2		2.92	2.17	1.67		2.7	1.58	0.46
3		2.98	2.26	1.82		2.92	2.22	1.27
4		3.02	2.38	1.92		2.96	2.33	1.41
5		3.08	2.42	1.96		2.99	2.48	1.6

Experiment 4. As experiment 3 indicates, the decomposition of hydrogen peroxide was promoted by the presence of fuchsine. To see whether this surplus of decomposition was caused by the use for the oxidation of fuchsine only, or the catalytic decomposition of hydrogen peroxide was also promoted, the amount of oxygen evolved by the catalytic decomposition of hydrogen peroxide was determined gasometrically.

0.1 gm. of well washed iron caolin, prepared as above mentioned, was treated with 1 cc. of 0.5% H_2O_2, 0.2 cc. of M/160 $FeCl_3$ in one of the gasometric flasks, while the same amount of

it was treated with 1 cc. of 0.5% H_2O_2 containing 0.04% fuchsine, 0.2 cc. of M/160 $FeCl_3$ in the other gasometric flask. At the definite interval, the reaction was checked by the addition of 2 cc. of H_2SO_4 (1 : 8). The amount of oxygen evolved by catalytic decomposition and the amount of gas produced from the rest of H_2O_2 by $KMnO_4$ were determined. The result is given in the following table.

TABLE XXI.

Nature of the mixture				Amount of oxygen		
Iron caolin	0.5% H_2O_2	M/160 $FeCl_3$	Fuchsine	Evolved by catalisation after 3h.	Evolved from rest H_2O_2 after 3h.	Total volume
gm.	*cc.*	*cc.*	*%*	*cc.*	*cc.*	*cc.*
0.1	1	0.2	0	1.36	0.44	1.8
0.1	1	0.2	0.04	1.49	0.14	1.63

As is indicated in the table, the catalytic decomposition of hydrogen peroxide is by the presence of fuchsine unquestionably promoted.

Experiment 5. The amount of iron adsorbed by caolin in experiment 3 was determined and found as follows (Zimmermann and Rheinhardt's method).

TABLE XXII.

Number of treatment of iron caolin	Amount of Fe expressed in terms of cc. of N 50 $KMnO_4$ used.		
	In 10 cc. of M/160 $FeCl_3$ used	In 10 cc. of filtrate	In 0.5 gm of washed ferric caolin
1	1.73	1.03	0.58
2		1.03	1.16
3		1.03	1.7
4		1.03	2.35
5		1.03	3.0
6		1.03	3.44

As can be seen from the above tables, the repeated treatment of caolin with iron solution acts unfavorably for the catalytic activity of iron salt on hydrogen peroxide in spite of the large quantity of iron which is adsorbed to caolin. This is apparently due to the deformation of the surface of caolin by the adsorption of hydrolysed iron hydroxide. The increase of non ionized iron hydroxide, so long as it does not dissolve, does not promote the activity of the decomposition of hydrogen peroxide by iron chloride solution.

Experiment 6. The oxidative activity of iron salt is promoted by the presence of a certain amount of caolin. This property of caolin is more marked when the latter is previously treated once with iron chloride (iron-caolin), while in caolin treated several times this promoting nature decreases. This relation may be illustrated by the following experiment.

To each 0.5 gm. of caolin, caolin once treated with $FeCl_3$ and caolin similarly treated five times, all of which were washed thoroughly until the reaction of Fe ion in the wash water had entirely disappeared, 4 cc. of 0.5% H_2O_2 containing 0.05% fuchsine and 1 cc. of M/100 $FeCl_3$ were added, and the mixture was allowed to stay at the room temperature (30°C). The colour changed in each of test-tubes was followed up and the result is shown in the following table.

TABLE XXIII.

Nature of caolin	Decolourized after the lapse of
0	16 hours
Caolin	5 hours
Iron caolin (once treated)	1 ½ hours
Iron caolin (treated 5 times)	2 ½ hours

IV. The Influence of Albumin, Lecithin and Cephalin on the Catalytic Activity of Iron Chloride on Hydrogen Peroxide.

A. Experiment with albumin.

The albumin used was prepared from the white of new-laid eggs by Hopkin's method, recrystallized four times from ammonium sulphate solution, dialysed for one week against tap water, 2 weeks against distilled water and finally 48 hours by the electro-endosmotic arrangement. The salt-free 2% albumin solution thus prepared which has the isoelectric point at pH = 4.9 was used as the stock solution and gives in itself no influence on hydrogen peroxide. This is proved by the following experiment.

To 1 cc. of varying concentration of albumin solution in a number of Erlenmeyer flasks were added 5 cc. of 0.2% H_2O_2 and directly after the mixing and 3 hours thereafter 5 cc. of H_2SO_4 (1 : 4) were added and the amount of H_2O_2 was titrated with N/10 $KMnO_4$.

The result is given in the following table.

TABLE XXIV.

Conc. of albumin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of	
	0	3h.
2	5.39	5.39
2/5	5.39	5.39
2/25	5.39	5.39
2/125	5.39	5.39
2/625	5.39	5.39
2/1250	5.39	5.39
0	5.39	5.39

Experiment 1. In this experiment the influence of albumin on the activity of iron chloride upon hydrogen peroxide was studied.

1. To 1 cc. of M/80 $FeCl_3$ solution in a number of Erlenmeyer flasks were added 1 cc. of varying concentration of albumin solution and 5 cc. of 0.2% H_2O_2 solution (pH=4.9). At the end of 30 m., 1 h., and 2 h., 5 cc. of H_2SO_4 (1 : 8) were added to the flasks one after another and H_2O_2 remained undecomposed in it was estimated by titration with N/10 $KMnO_4$. (Table XXV.)

TABLE XXV.

Endconc. of $FeCl_3$=M/560. Room temp. 25°C.

Albumin solution added		Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
Conc. (%)	pH	0	30 m.	1 h.	2 h.
2	4.8		5.07	4.93	4.76
2/5	4.8		5.07	4.87	4.54
2/25	4.9		4.33	3.3	1.81
2/125	4.9		2.61	1.43	0.48
2/625	4.9		2.15	0.98	0.54
2/1250	4.9		2.02	1.02	0.54
0	4.9	5.59	2.02	1.02	0.54

2. To 4 cc. either of M/160 or of M/320 $FeCl_3$ solution in a number of Erlenmeyer flasks were added 4 cc. of varying concentration of albumin solution and finally 20 cc. of 0.2% H_2O_2. After a definite length of time 5 cc. of the mixture were removed for analysis of H_2O_2. (Table XXVI and XXVII.)

TABLE XXVI.

Endconc. of $FeCl_3$ = M/1120. Room temp. 25°C.

Conc. of albumin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1 h.	2 h.	3 h.
2/25		3.12	2.6	2.2
2/50		2.58	1.79	1.28
2/100		2.08	1.26	0.8
2/200		1.8	**1.03**	**0.66**
2/400		**1.78**	1.13	0.87
2/800		1.98	1.5	1.25
2/1600		2.13	1.77	1.56
2/3200		3.15	1.85	1.63
2/6400		2.15	1.92	1.72
0	3.77	2.24	1.92	1.72

TABLE XXVII.

Endconc. of $FeCl_3$ = M/2240. Room temp. 25°C.

Conc. of albumin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1 h.	2 h.	3 h.
2/25		3.65	3.5	3.4
2/50		3.56	3.28	2.98
2/100		3.44	3.0	2.62
2/200		3.3	**2.81**	**2.36**
2/400		**3.25**	2.93	2.5
2/800		3.33	3.13	2.96
2/1600		3.38	3.19	3.06
0	3.77	3.38	3.19	3.06

From the foregoing three tables it may be deduced that the addition of a certain amount of albumin solution at its isoelectric point to the system of $H_2O_2 + FeCl_3$ acts favorably on the catalytic activity of iron, while a too large amount retards the reaction very markedly. It is worth while to mention that on addition of albumin solution to the iron chloride solution the yellowish-brown colour of iron solution fades to a faint yellowish-white and the solution becomes a little opaque. Such a solution remains yellow in colour for 2–3 hours, while other solutions turn to brown in the course of time.

Experiment 2. It has shown in experiment 1 that the addition of a certain amount of isoelectric albumin solution favours the catalytic activity of iron on hydrogen peroxide. In the following experiments I have studied the influence of the addition of albumin at an other acidity than pH=4.9.

Since the buffer solutions, ordinarily used, do not serve well owing to the formation of insoluble salt with iron chloride, so the variant acidities were brought about by merely adding hydrochloric acid or sodium hydroxide. In this way 0.2% hydrogen peroxide solutions of different acidity were prepared, and their acidities were checked by the indicator method described by Clark. Using these solutions the similar experiments as above were performed, the results of which are indicated in the following table.

TABLE XXVIII.

pH of the H_2O_2 solution = 3.
Endconc. of $FeCl_3$ = M/1120. Room temp. 25°C.

Conc. of albumin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of		
	0	1 h.	2 h.
2/25		2.96	2.3
2/50		2.3	1.4
2/100		1.71	0.88

Conc. of albumin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of		
	0	1 h.	2 h.
2/200		1.41	0.67
2/400		1.22	0.55
2/800		1.12	0.48
2/1600		1.05	0.45
2/3200		1.02	0.45
0	3.77	1.02	0.45

TABLE XXIX.

pH of the H_2O_2 solution = 3.
Endconc. of $FeCl_3$ = M/2240. Room temp. 25°C.

Conc. of albumin added %	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1 h.	2 h.	3 h.
2/25		2.53	3.29	2.95
2/50		2.34	2.83	2.25
2/100		3.05	2.31	1.57
2/200		2.77	1.9	1.16
2/400		2.63	1.75	1.01
2/800		**2.5**	**1.59**	**0.87**
2/1600		2.52	1.64	0.99
2/3200		2.52	1.71	1.25
0	3.77	2.52	1.75	1.27

TABLE XXX.

pH of the H_2O_2 solution = 9.
Endconc. of $FeCl_3$ = M/1120 Room temp. 25°C.

Conc. of albumin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of				
	0	1 h.	2 h.	3 h.	4 h.
2/25		3.42	2.96	2.8	2.61
2/50		3.31	2.65	2.4	2.15
2/100		3.11	**2.27**	**1.97**	**1.69**
2/200		3.04	2.46	2.24	2.0
2/400		3.04	2.57	2.42	2.25
2/800		3.04	2.66	2.53	2.4
0		3.04	2.66	2.53	2.4
0 ($FeCl_3$=0)	3.77				3.77

It is evident from the above experiment that at the higher acidity the favorable concentration of albumin seems to shift to the more dilute solution, while at the lower acidity the more concentrated solution accelerates the catalytic activity of iron on hydrogen peroxide.

Experiment 3. It has been shown above that the addition of albumin is the most favorable when 0.01% albumin solution is added to the same volume of M/160 iron chloride solution. Taking this ratio as always constant, the various acidities of the system were prepared by using the hydrogen peroxide solutions of different hydrogen ion concentration. The catalytic activity of such a system was determined, while at the same time the equivalent test without the addition of albumin was performed.

The result is given in the following table.

TABLE XXXI.

Endconc. of $FeCl_3$ = M/1120
Endconc. of albumin = 1/700%. Room temp. 25°C.

pH of H_2O_2 sol. used	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of							
	With albumin				Without albumin			
	0	1 h.	2 h.	3 h.	0	1 h.	2 h.	3 h.
3		2.18	1.16	0.66		1.4	0.7	0.46
4.9		2.43	1.5	0.95		2.26	1.87	1.66
9.8		3.31	2.98	2.63		3.17	2.89	2.67
9.8 ($FeCl_3$ = 0)	3.77			3.77	3.77			3.77

As will be seen from the table the addition of albumin may accelerate the catalytic activity of iron salt only at the acidity of neighbourhood pH = 4.9, while there is no favorable effect observable at a higher or lower acidity.

Experiment 4. In this experiment the comparison was made on the effectiveness of freshly prepared iron albumin mixture and old one.

The iron albumin obtained as above mentioned was allowed to remain at room temperature for several periods of time and its activity on hydrogen peroxide was tested in the same way as experiment 3 and the value obtained was compared with that of blank experiment without albumin.

TABLE XXXII.

Endconc. of $FeCl_3$ = M/1120.
Endconc. of albumin = 1/700%

Age of iron albumin.	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of						
	With iron albumin				Without albumin		
	0	1 h.	2 h.	3 h.	1 h.	2 h.	3 h.
day fresh	3.57	2.1	1.48	0.95	2.3	1.95	1.71
2		3.06	2.45	1.9	2.82	2.26	1.85
3		3.18	2.65	2.12	2.92	2.4	1.98

The accelerating action of iron-albumin-mixture is therefore only varied in the day of preparation, while even two days later the efficiency is decreased and the difference between it and the system lacking albumin becomes much blurred.

B. Experiment with lecithin and cephalin.

In the foregoing paragraphs it has been shown that the albumin at a certain concentration accelerates the catalytic activity of iron on hydrogen peroxide and it may be well assumed that the surface phenomenon, viewed from the results obtained under different experimental conditions, is playing here a special role. If it be really the case, other colloidal substance will display the same relation. Having this in mind, I proceeded to test the effect of the presence of lecithin and cephalin on the catalytic power of iron salt.

Material.

Lecithin. The lecithin used in my experiment was prepared from the yolk of new-laid eggs and the brain of ox by the method of Maclean (1918) and 1% stock solution was made dissolving it into distilled water.

Cephalin. The cephalin used was prepared from the ox brain following the method described by Koganei (1923). 1% stock solution was made dissolving it into distilled water.

Lecithin and cephalin thus prepared showed in itself no evidence of catalytic power on hydrogen peroxide and it is also hardly acted upon in turn under the conditions obtaining in the following experiment by potassium permanganate, so that it is not necessary to take any care of its presence in the determination of catalytic activity of iron salt on hydrogen peroxide.

Experiment. The method of examining the effect of lecithin and cephalin on the catalytic power of iron remained just the same as in the case of albumin.

The results are given in the following tables.

TABLE XXXIII.

Egg lecithin.

Endconc. of $FeCl_3$ = M/560. Room temp. 25°C.

Conc. of lecithin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	40m.	1h.	1½h.
1/5		2.4	1.72	1.22
1/10		1.7	1.15	0.72
1/20		1.58	0.96	0.6
1/40		1.53	**0.96**	**0.54**
1/80		1.5	0.98	0.56
1/160		1.5	1.06	0.56
1/320		1.48	1.06	0.64
0	5.39	1.48	1.06	0.64

TABLE XXXIV.

Egg lecithin

Endconc. of $FeCl_3$ = M/1120. Room temp. 24°C.

Conc. of lecithin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
1/5		3.59	2.88	2.23
1/10		3.18	2.31	1.81
1/20		2.92	1.93	**1.47**
1/40		**2.88**	**1.85**	**1.5**
1/80		2.95	2.00	1.7
1/160		2.95	2.18	1.88
1/320		3.27	2.67	2.17
1/640		3.29	2.7	2.3
1/1280		3.41	2.8	2.3
1/2560		3.44	2.87	2.45
0	3.89	3.44	2.95	2.58

TABLE XXXV.

Egg lecithin

Endconc. of $FeCl_3$ = M/2240 Room temp. 24°C.

Conc. of lecithin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
1/5		3.35	3.12	2.98
1/10		3.17	2.78	2.59
1/20		**3.14**	**2.7**	**2.46**
1/40		3.24	2.85	2.62
1/80		3.34	3.1	2.92
1/160		3.41	3.21	3.11
1/320		3.44	3.28	3.21
0	3.89	3.46	3.28	3.21

TABLE XXXVI.

Ox brain lecithin

Endconc. of $FeCl_3$ = M/1120. Room temp. 25°C.

Conc. of lecithin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
1/5		2.53	1.92	1.55
1/10		2.22	1.59	1.18
1/20		2.13	**1.45**	**1.06**
1/40		**2.08**	1.5	1.18
1/80		2.13	1.63	1.37
1/160		2.22	1.78	1.54
1/320		2.26	1.85	1.62
1/640		2.3	1.96	1.75
0	3.89	2.44	2.08	1.84

TABLE XXXVII.

Cephalin.

Endconc. of $FeCl_3$ = M/560. Room temp. 25°C.

Conc. of cephalin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	30′	1h.	1½h.
1/5		2.78	0.91	**0.54**
1/10		**2.28**	**0.84**	0.57
1/20		2.28	0.87	0.57
1/40		2.38	0.91	0.57
1/80		2.48	0.97	0.57
1/160		2.48	1.12	0.62
0	5.39	2.48	1.12	0.62

TABLE XXXVIII.

Cephalin.

Endconc. of $FeCl_3$ = M/1120. Room temp. 25°C.

Conc. of cephalin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
1/5		2.0	1.28	**0.8**
1/10		**1.88**	**1.18**	**0.87**
1/20		2.0	1.45	1.17
1/40		2.06	1.54	1.26
1/80		2.15	1.62	1.32
1/160		2.27	1.77	1.47
1/320		2.32	1.85	1.54
1/640		2.35	1.87	1.57
0	3.89	2.35	1.87	1.57

TABLE XXXIX.

Endconc. of $FeCl_3$ = M/2240. Room temp. 25°C.

Conc. of cephalin added (%)	Amount of H_2O_2 present (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of			
	0	1h.	2h.	3h.
1/5		**3.07**	**2.57**	**2.24**
1/10		3.11	2.75	2.49
1/20		3.28	3.02	2.83
1/40		3.32	3.14	2.99
0	3.89	3.32	3.14	2.99

We can see from the tables that the same relation is prevailing here as was present in the case of albumin. At a

certain concentration lecithin and cephalin, that is to say, promote the catalytic activity of iron strongly, while its larger amount tends to retard the activity and the case is the same with caolin.

V. Influence upon each other by Iron Chloride and Hemase; Peroxidase and Hemase.

In the domain of cellular physiology, much has been said hitherto about enzymes which deal with the oxidative process. Oxidase and peroxidase system on the one hand, and Wieland's dehydrogenase on the other, both play the important role. As to the function of catalase, Burge persists that its amount is parellel with the activity of biological oxidations, but Stehle (1919) failed to find any such paralellism.

As iron chloride has both the catalytic and oxidative power, it is quite interesting to know how peroxidase and catalase on the one hand and iron chloride and enzymes on the other influence each other.

The author of this article made some experiments and the results of this study are given in the following paragraphs.

A. The mutual effect of their presence displayed on their activity between hemase and iron chloride.

As hemase and iron chloride both decompose the hydrogen peroxide, it is quite possible that they may act upon each other either favorably or unfavorably, or remain indifferently. I have examined their mutual influence and am going to describe them in the following paragraphs.

Hemase used in this experiment was prepared by Ostwald-Senter's method from 200 cc. of defibrinated blood of horse. It was further purified by centrifuging it in 50% alcohol 5 times. The yield was 30 gm. of reddish yellow powder. 1.5 gm. of the powder was suspended in 10 cc. of distilled water and kept in an ice chest for 24 hours and then filtered. The yellow filtrate

thus prepared was used as the stock solution of hemase. 1 cc. of such a solution contained 0.3 mg. of nitrogen and was used in the experiment after it had been diluted with water properly.

1. The effect of the presence of iron chloride on the activity of hemase on hydrogen peroxide.

To 4 cc. of 1/40 hemase and 4 cc. of $FeCl_3$ of varying concentration in a number of Erlenmeyer flasks were added 20 cc. of 0.2% H_2O_2. After 1 h., 2 h. and 3 hours, 5 cc. of the mixture were pipetted out into Erlenmeyer flask, 4 cc. of H_2O_2 (1 : 4) were added and then the amount of H_2O_2 was estimated by titration with N/10 $KMnO_4$.

The result is given in the following table.

TABLE XL.

Room temp. 24.5° C.

Initial amount of H_2O_2 corresponds to 4.49 cc. of N/10 $KMnO_4$ used.

Conc. of $FeCl_3$ sol. added	Amount of H_2O_2 decomposed (expressed in terms of cc. of N/10 $KMnO_4$ used) after the lapse of					
	1h.		2h.		3h.	
	$FeCl_3$ alone	$FeCl_3$ + hemase	$FeCl_3$ alone	$FeCl_3$ + hemase	$FeCl_3$ alone	$FeCl_3$ + hemase
0		1.72		1.72		1.72
1/2560	0.02	1.68	0.03	1.68	0.04	1.68
1/1280	0.04	1.59	0.07	1.59	0.09	1.59
1/640	0.11	1.52	0.16	1.52	0.19	1.52
1/320	0.47	1.12	0.59	1.44	0.67	1.65
1/160	2.07	2.46	2.54	3.46	2.77	3.86
1/80	3.84	3.96	4.19	4.29		

As it will be seen in the table, the presence of iron salt retards the action of hemase very clearly.

2. *The effect of the presence of hemase on the activity of iron chloride on hydrogen peroxide.*

As iron salt has the nature of both catalytic and oxidative activities, the effects of hemase on both activities were examined.

a. The effect of hemase on the catalytic activity of iron chloride.

To 4 cc. of M/160 $FeCl_3$ and 4 cc. of varying concentration of hemase solution in a number of Erlenmeyer's flasks were added 20 cc. of 0.4% H_2O_2 and with the addition of 4 cc. of distilled water, the total volume was made to 32 cc. After the lapse of 1 h., 2 h. and 3 hours, 5 cc. of the mixture were pipetted out for analysis.

As I saw in a preliminary test, the addition of a certain amount of hemase solution promotes the catalytic activity of iron just as it has been observed in the case of albumin. I have also examined the effect of addition of 4 cc. of albumin (1 cc. = 0.0165 mg.) in the system of the above experiment in place of water using the same procedure.

In the following table the results of such experiments are given.

TABLE XLI.

Conc. of $FeCl_3$ added = M/160. Room temp. 24.5°C.
N in 1 cc. of albumin solution added = 0.0165 mg.
N in 1 cc. of 1/20 hemase solution added = 0.015 mg.

Nature of mixture		Amount of H_2O_2 decomposed in terms of cc. of N/10 $KMnO_4$ used		
		1 hour		
Conc. of hemase added	N in 1 cc. of hemase (mg.)	Hemase only	Hemase + $FeCl_3$	Hemase + $FeCl_3$ + albumin
0	0	0	2.05	**2.42**
1/160	0.00188	0.23	2.16	2.35
1/80	0.00375	0.46	2.38	2.22
1/40	0.0075	1.08	**2.48**	2.14
1/20	0.015	2.43	**2.46**	2.05
1/10	0.03	5.06	2.53	2.51

Nature of mixture		Amount of H_2O_2 decomposed in terms of cc. of N/10 $KMnO_4$ used		
Conc. of hemase added	N in 1 cc. of hemase (mg.)	2 hours		
		Hemase only	Hemase + $FeCl_3$	Hemase + $FeCl_3$ + albumin
0	0	0	2.6	**4.06**
1/160	0.00188	0.23	2.88	3.88
1/80	0.00375	0.46	3.34	3.74
1/40	0.0075	1.11	3.8	3.47
1/20	0.015	2.48	**3.83**	3.25
1/10	0.03	5.28	3.48	3.25
		3 hours		
0	0	0	2.94	**4.92**
1/160	0.00188	0.23	3.26	4.78
1/80	0.00375	0.46	3.82	4.62
1/40	0.0075	1.11	4.48	4.3
1/20	0.015	2.52	**4.56**	4.0
1/10	0.03	5.34	4.13	3.56

As this table indicates, the presence of hemase acts favorably at a certain concentration just as it did in the case of albumin. If we compare the amount of nitrogen contained in both cases there is a fair coincidence. We can therefore assume that the hemase does not exert any specific influence on the catalytic activity of iron salt on hydrogen peroxide.

b. The effect of hemase on the oxidative activity of iron chloride.

Into a series of test tubes of the same calibre 1 cc. of 1/160 $FeCl_3$ and 1 cc. of varying concentration of hemase and finally 10 cc. of 2% H_2O_2 containing 0.01% methylenblue were measured.

After each of definite intervals the change of colour was compared with the scale which was made by the addition of varying amount of iron chloride to the methylenblue solution, the same as the experiment, and the oxydative power was estimated.

The result is given in the following table.

TABLE XLII.

Conc. of hemase added	The oxydative power of iron chloride after the lapse of (expressed in %)		
	30 m.	1h.	2h.
	%	%	%
1/5	70	70	70
1/10	80	80	80
1/20	80	80	100
1/40	110	120	120
1/80	110	110	110
1/160	110	110	110
1/320	110	110	110
1/640	110	110	110

Hemase has therefore just the same effect on catalytic and oxidative activity of iron chloride.

B. The mutual effect of their presence played on their activity between hemase and peroxidase.

1. The effect of the presence of peroxidase on the catalytic activity of hemase.

Material

Peroxydase. The peroxydase used in this experiment was prepared from 1 kgm. Wasabia japonica, Matsumura according to Bach and Chodat's method. After purification was repeated three times 1.4 gm. of peroxidase was obtained as white powder.

Pyrogallol solution. Pyrogallol (Merck) used was purified free from gallic acid and salt by recrystalization. Its 4% solution in distilled water was prepared just previous to its use.

Experiment 1. The effect of the presence of peroxydase on hemase was studied from the change of volume of oxygen evolved by the action of hemase on hydrogen peroxide in its presence.

4 cc. of 2% pyrogallol solution and varying quantity of peroxidase dissolved in 1 cc. of water, were measured into the flask of gasometric apparatus and kept in a thermostat at 30°C., and then 1 cc. of 1/40 hemase and 5 cc. of 0.2% H_2O_2 were added. After 30 minutes, the reaction was checked by the addition of 2 cc. of H_2SO_4 (1 : 8). The flask was shaken gently for five minutes and the oxygen evolved was measured.

As when the peroxidase acts on pyrogallol + H_2O_2 it evolves a certain amount of oxygen, it was necessary to determine its amount by conducting the same procedure as above only without addition of hemase.

The result is given in the following table.

TABLE XLIII.

Nature of mixture				Volume of oxygen evolved after 30′ at 30°C.	
0.2% H_2O_2	4% Pyrogallol	1/40 Hemase	Amount of peroxidase	Hemase + peroxidase	Peroxidase alone
cc.	*cc.*	*cc.*	*gm.*	*cc.*	*cc.*
5	2	1	0	0.98	0
5	2	1	0.0025	0.62	0.06
5	2	1	0.005	0.54	0.1
5	2	1	0.01	0.48	0.17
5	2	1	0.02	0.6	0.3

Experiment 2. The effect of peroxidase on the hemase activity in the system hemase + H_2O_2 + α-naphtol was also studied with peroxidase obtained from Arctium lappa L. by the method of Bach and Chodat.

The system consisted of 5 cc. of 0.1% H_2O_2, 1 cc. of 1/40 hemase and 5 mg. of α-nophtol which was suspended in 1 cc. of of 1 mol alcohol and the whole procedure remained as before.

The result is given in the following table.

TABLE XLIV.

Nature of mixture				Volume of oxygen evolved after 30′ at 30°C.	
0.1% H_2O_2	Quantity of α-naphtol suspended in 1 cc. of 1 mol alcohol	1/40 hemase	Peroxidase	Hemase + peroxidase	Peroxidase alone
cc.	*gm.*	*cc.*	*gm.*	*cc.*	*cc.*
5	0.05	1	0	1.7	0
5	0.05	1	0.005	1.5	0
5	0.05	1	0.01	1.2	0

The foregoing two tables indicate that the peroxidase undoubtedly retards the activity of hemase.

2. The influence of hemase on the activity of peroxidase.

We have seen in the above experiments that peroxidase has an unfavorable influence on the action of hemase. The reverse influence, i. e., the effect of hemase on the activity of peroxidase is quite interesting, as we can predict two possible cases here — a favorable and an unfavorable one. As I have already pointed out, many previous authors have shown that the excessive amount of hydrogen peroxide is very poisonous for the activity of peroxidase. If we examine the activity of peroxidase with a large amount of hydrogen peroxide, the presence of hemase will act favorably by decomposing the excess of hydrogen peroxide, while in case the hydrogen peroxide present is not enough, the diminution of it caused by the presence of hemase will act entirely unfavorably. To elucidate this reaction, I have made the following experiments.

Experiment. Into each Erlenmeyer flask containing 10 cc. of H_2O_2 were added 2 cc. of 4% pyrogallol solution, 1 cc. of 1% peroxidase and a varying amount of hemase solution, such as 1 cc., 2 cc., 3 cc., 4 cc., 5 cc., and 6 cc. and with the addition of water the total volume was made to 20 cc. The mixture was allowed to stand at room temperature for 12 hours. The

amount of purpurogallin obtained in this condition was determined by titration with N/50 $KMnO_4$ according to Bach and Chodat's method.

1 cc. of hemase used decomposes 1.2 cc. of 0.2 % H_2O_2 in the presence of pyrogallol under these conditions.

TABLE XLV.

Nature of mixture				Amount of purpurogallin in terms of N/50 $KMnO_4$ used
0.2% H_2O_2	4% Pyrogallol	Peroxidase	Amount of hemase.	
cc.	*cc.*	*gm.*	*cc.*	*cc.*
10	2	0	0	0
10	2	0	1	0
10	2	0.01	1	1.27
10	2	0.01	2	2.87
10	2	0.01	3	3.88
10	2	0.01	4	3.90
10	2	0.01	5	4.2
10	2	0.01	6	5.2

As can be seen from the table, the amount of purpurogallin increases when hemase is present. That it is mainly due, at least, to the diminution of hydrogen peroxide is quite reasonable to suppose from the view of two points.

Firstly, when peroxidase and pyrogallol in the same amount as in the above experiment were added to hydrogen peroxide of the decreasing amount (10, 8, 6.8, 5.6, 4.4, 3.2), the production of purpurogallin was increased accordingly as is shown in the following table.

TABLE XLVI.

Nature of mixture			Amount of purpurogallin in terms of cc. of N/50 $KMnO_4$ used
0.2% H_2O_2	4% Pyrogallol	Peroxydase	
cc.	*cc.*	*gm.*	*cc.*
10	2	0.01	0.8
8	2	0.01	1.67
6.8	2	0.01	2.67
5.6	2	0.01	4.4
4.4	2	0.01	6.15
3.2	2	0.01	9.6

Secondly, when 1 or 3 cc. of hemase was added to the mixture of 5 cc. of 2% H_2O_2 and 2 cc. of 4% pyrogallol, hydrogen peroxide was decomposed nearly to the amount which would give rise to the amount of purpurogallin when acted on by peroxidase on the condition maintained in the above experiments.

TABLE XLVII.

Nature of mixture			Amount of oxygen evolved in terms of 0.2% H_2O_2 after 30′ at 30°C.
0.2% H_2O_2	4% Pyrogallol	Hemase added	
cc.	*cc.*	*cc.*	*cc.*
5	2	1	1.2
5	2	3	3.2

In the above experiment the amount of hydrogen peroxide was quite large and the retarding action of hemase could not be observed. I was, however, able to get an evidence of both the promoting and the retarding action of hemase on oxidizing enzyme with another sample of peroxidase, which is not very pure and contains a little of oxidase, but it was possible to see the relation

clearly. The procedure of the experiment was quite the same as above mentioned.

The results are given in the following table.

TABLE XLVIII.

Nature of mixture			Amount of purpurogallin in terms of N/50 $KMnO_4$		Colour of precipitate
0.2% H_2O_2	4% Pyrogallol	Peroxidase	Peroxidase alone	Peroxidase + hemase	
cc.	*cc.*	*gm.*	*cc.*	*cc.*	
4	2	.01	2.0	2.2	brown
3	2	.01	2.4	2.45	„
2	2	.01	3.3	4.16	„
1	2	.01	14.8	13.2	dark brown
0.5	2	.01	10.8	9.4	„
0	2	.01	7.8	7.8	„

As is shown in the table, the amounts of hydrogen peroxide 4 cc., 3 cc. and 2cc. are too large for the amount of peroxidase 0.01, and the addition of hemase promotes the production of purpurogallin, while in the smaller amount of hydrogen peroxide the presence of hemase retards the action of peroxidase. In case there is no hydrogen peroxide present, hemase naturally neither promotes nor accelerates the production of purpurogallin.

Summary

1. A new micro-gasometric apparatus is devised.

2. Among the chloride of several heavy metals the catalytic activity of iron is most effective, while copper comes next, and salts of other metals remain very weak in their effectiveness.

3. This catalytic activity seems to be due to the presence of metallic ion.

4. The catalytic activity of iron chloride weakens therefore proportionately with the increase of hydrolysis.

5. The oxidative activity of salt of iron and other metals is almost parallel with their catalytic activity.

6. The presence of caolin, at a certain amount, promotes both the catalytic and oxidative activity of iron salt. The excess of caolin, however, retards the activity of iron. The repeated treatment of caolin with iron chloride lessens the effectiveness of caolin in promoting the activity of iron salt probably owing to the deformation of the surface.

7. The addition of a certain amount of isoelectric albumin to the system of $H_2O_2 + FeCl_3$ accelerates the catalytic action of iron salt on hydrogen peroxide, while the optimum concentration, at the other acidity than the isoelectric point, shifts to either direction.

8. The same relation as that of albumin is also observable in case of the addition of lecithin and cephalin.

9. The presence of iron salt retards the action of hemase very clearly.

10. The presence of hemase acts favourably on the catalytic activity of iron salt at a certain concentration just as in the case of albumin. This effectiveness of hemase is found to be due to its albumin content.

11. The presence of peroxidase retards the activity of hemase.

12. The presence of hemase promotes the activity of peroxidase and oxidase when the amount of hydrogen peroxide present is large, while in the case where the amount of hydrogen peroxide is not sufficiently large for the action of peroxidase, it acts retardingly.

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ON THE BIOCHEMICAL STUDY OF THE RIPENING OF THE KAKI-FRUIT. V.*

By

SHIGERU KOMATSU AND MOTARO ISHIMASA.

(*From the Biochemical Laboratory, Kyoto Imperial University.*)

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In the second article on this subject, one of the authors and H. Ueda (1923) studying the daily change in the carbohydrates and shibu contents of the sweet kaki-fruit grown in Shirakawa, Kyoto, have reached the conclusion that as the fruit becomes ripe, the reducing sugar-content increases, and the soluble shibu is largely converted into an insoluble form.

The present investigation was, therefore, undertaken to ascertain whether the same chemical transformation in the constituents of the fruit of the astringent variety would occur in the course of ripening.

I. The Ripening and Growth of Astringent Kaki-Fruit.

The samples used in this experiment were two strains of the astringent variety grown in different places; one is "marugaki" in Shirakawa and the university ground, and the other "daishiro" in Tanaka, Kyoto.

The sampling of the fruit and the analytical methods employed were the same as those described in the former article. The day chosen for sampling was fairly bright and sunny, as it was in the previous experiment.

* The expenses of this investigation were shared by the Government Department of Education.

In the determination of simple sugars, the water extract was prepared boiling the pulp with some quantity of calcium carbonate to avoid inversion of cane sugar by the action of organic acids present in the fruit during preparation of the extract, and the results of the analysis were compared with those resulting from the extract prepared by the usual process, as will be seen in the table.

Pectin content in the fruit was determined by the usual process, and also by the precipitation of the water extract of the fruit with calcium chloride solution, proposed by M. H. Carré and D. Haynes (1922).

The analytical results calculated im grammes per fruit were shown in tables I, IV, VII, with the condition of the fruit on the tree at the time of sampling. In tables II, V, VIII, percentage results in the pulp were shown and the figures given in tables III, VI, IX. were percentages in dry pulp.

The following generalisations were made from the experimental results mentioned above :

1. The actual weight of the fruit increased at the rate of one gramme per diem.

2. During growth of the fruit the amount of total sugar increased and that of polysaccharides, including cellulose, on the contrary, decreased.

3. The quantity of sucrose increased from 7 per cent on July 26. in " marugaki," Shirakawa, to 26 per cent, the maximum on Sep. 18., and then fell to 12 per cent on November 6.

4. When the fruit attained maturity, the amount of the reducing sugar showed a sudden increase, and, contrary-wise, that of sucrose decreased together with the soluble shibu-content, this increase in the content of reducing sugar may be explained by assuming an inversion of sucrose into reducing sugars.

5. The fructose-glucose-ratio in the fruit approaches with time that of the invert sugar, owing to the increase in fructose as due to hydrolysis of sucrose, the glucose produced by that

reaction having been partly used in respiration.

6. The inversion of sucrose having no numerical relation between the velocity of inversion and the acidity of the fruit extract, it seems proper to ascribe it to the action of invertase in the fruit.

Although the transformation of the soluble shibu in the astringent fruit into an insoluble one, was very slow compared with the rate of change in the sweet kaki, when the fruit reached maturity, the characteristic softening of the pulp began, accompanied by marked lessening in the content of the soluble shibu, cane sugar and acids, and on the other hand by increase of the reducing sugar-content, even as similar changes were already observed in these constituents of the sweet kind.

The authors, therefore, with regard to the nature of the ripening of the kaki-fruit, including both sweet and astringent varieties insist upon the opinion which they proffered in the second article, namely, the reducing sugar-content increases and on the other hand the soluble shibu-content decreases.

One of the important factors in the ripening of the fruit as will be seen in the experimental results was the accumulation of enough reducing sugar to balance the unpleasant acrid taste due to the presence of organic acids and soluble shibu by the sweetness of the sugar but not to lead to an entire disappearance of the soluble shibu. As a matter of fact, the following proportions of reducing sugar, soluble shibu and acid contents of the ripe fruit were observed to exist:

sugar 10 : soluble shibu 0.2 : acid 0.1

It was also an important factor in maintaining the sweetness of the fruit that the fructose-glucose-ratio in the reducing sugar, keeping the definite proportions above mentioned among the three essential constituents, sugar, shibu and acid, should not allowed to exceed 1.2.

It was very strange that the nitrogenous matter in the astringent fruit increased gradually with time, while that in the

sweet decreased.

The fruit of the astringent variety was generally used, in Japan, as a souce of fruit jellies. Some advantages arising out of the use of the fruit in jelly-making depend upon the presence therein, in high proportions, of pectin.

The chemical change of the constituents of the picked fruit during storage at room temperature, differed somewhat in detail from that of the fruit on the tree, especially as concerns the content of cane suger and shibu.

"Saijô" grown in Onomichi, Hiroshimaken, picked on Nov. 1. and analysed the next day and on the 9th., keeping it in the laboratory. During storage, the cane sugar and soluble shibu of the picked fruit as seen in the accompanying table, X, XI, XII, disappeared entirely by conversion of the former into the reducing sugars, and of the latter into an insoluble form, while the weight of marc showed a corresponding increase. Such facts seem to support the explanation with regard to the insolubility of shibu, during the period of ripening, that the shibu becomes associated with a second colloid such as protein or some other substance in the fruit, with which it forms an insoluble compound (Y. Kumagai and K. Tazaki, 1922; F. E. Lloyd, 1911).

Such a view of the insolubility of the shibu was already rejected by one of us and H. Ueda in the second article, and the true mechanism of its transformation by further study of its chemical nature, will be explained.

TABLE I.

"Marugaki" in Shirakawa.

gm. in per fruit.

Date	Total sugar		Reducing sugar		Cane sugar		Pectin		Tannin		Polysaccharide	Cellulose	Marc	Acid	Nitrogen	Ash	Water	Wt. per fruit.	Remarks
	I.*	II.*	I.*	II.*	I.*	II.*	I.°	II.°	I.°	II.°									
VII 26	1.28	1.30	1.03	1.02	0.25	0.34	0.63	—	0.44	—	0.16	0.19	1.14	0.12	0.015	0.13	25.5	38	
VIII 2	1.16	1.24	1.13	1.09	0.03	0.15	0.56	—	0.77	—	0.15	0.18	1.28	0.11	0.017	0.14	27.8	40	
9	2.36	1.77	1.63	1.69	0.73	0.87	0.96	0.90	0.72	0.72	0.32	0.25	1.93	0.11	0.002	0.16	41.8	61	
IX 4	5.11	4.07	2.60	2.51	2.12	1.56	1.59	1.48	1.51	1.08	0.37	0.37	2.61	0.14	0.023	0.30	57.1	84	Becomes yellow
11	5.21	5.08	2.74	2.60	2.47	2.48	1.18	0.78	1.08	1.28	0.53	0.41	2.70	0.14	0.031	0.31	60.8	88	
18	5.68	5.63	2.49	2.44	3.28	3.19	1.25	0.99	0.92	1.16	0.48	0.36	2.61	0.11	0.058	0.29	54.5	80	
X 3	8.40	8.40	6.17	6.14	2.23	2.34	1.19	0.99	1.13	1.18	0.64	0.37	3.07	0.11	0.061	0.30	66.4	105	
18	12.73	11.29	10.63	6.85	2.10	4.44	2.28	2.08	0.93	0.81	1.02	0.44	3.47	0.14	0.085	0.41	89.1	133	
XI 6	15.64	15.65	13.33	10.17	3.38	5.47	2.95	1.60	0.83	0.71	0.47	0.47	3.85	0.19	0.132	0.52	87.0	145	Yellowish orange, a little acrid taste.
21	24.74	25.20	20.54	25.20	4.20	0.00	2.09	1.55	0.56	0.42	0.51	0.55	4.56	0.23	0.231	0.56	127.6	198	Flesh softens, no acrid taste.

I.* Water extract by usual way.
II.* Water extract treating with $CaCO_3$.

I.° Used alcohol for ppt. pectin.
II.° Used $CaCl_2$-solution ppt. pectin.

TABLE II.

"Marugaki" in Shirakawa. Percentage in "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Marc	Polysaccharide	Cellulose	Nitrogen	Ash	Water
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°							
VII	26	4.266	4.50	2.39	3.39	0.83	1.12	2.08	—	1.44	—	0.41	4.46	0.52	0.64	0.051	0.41	84.3
VIII	2	3.518	3.78	3.43	3.37	0.08	0.46	1.69	—	2.33	—	0.33	3.90	0.45	0.55	0.02	0.42	84.9
	9	4.722	5.10	3.25	3.37	1.46	1.74	1.93	1.81	1.44	1.45	0.23	3.85	0.64	0.51	0.03	0.37	83.7
IX	4	6.686	5.65	3.68	3.56	3.01	2.21	2.26	2.10	2.14	1.53	0.20	3.69	0.53	0.48	0.03	0.43	80.7
	11	7.107	6.93	3.74	3.56	3.37	3.38	1.61	1.05	1.46	1.68	0.19	3.68	0.76	0.56	0.04	0.42	82.9
	18	8.627	8.42	3.72	3.65	4.90	4.77	1.87	1.48	1.37	1.65	0.16	3.90	0.72	0.54	0.09	0.43	81.4
X	3	10.211	10.31	7.50	7.45	2.71	2.86	1.44	1.21	1.41	1.43	0.14	3.86	0.77	0.44	0.07	0.36	80.7
	18	11.360	10.67	9.49	6.11	1.87	3.97	2.04	1.86	0.83	0.72	0.13	3.10	0.73	0.39	0.08	0.33	79.5
XI	6	13.436	12.52	10.67	8.14	2.71	4.38	2.67	1.28	0.67	0.58	0.16	3.16	0.38	0.37	0.11	0.49	77.7
	21	15.09	15.37	12.52	15.37	2.56	0.00	1.28	0.94	0.34	0.25	0.14	2.84	0.31	0.34	0.14	0.34	77.8

TABLE III.

"Marugaki" in Shirakawa. Percentage in dry "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Marc	Polysaccharides	Cellulose	Nitrogen	Ash	Glucose-fructose ratio	
		I*	II*	I*	II*	I*	II*	I°	II°	I	II°							I	II
VII	26	26.95	28.61	21.68	21.52	5.27	7.066	13.22	—	9.19	—	2.90	28.34	3.33	4.03	0.32	2.83	1.9	1.7
VIII	2	23.13	24.41	22.71	21.91	0.42	2.495	11.21	—	15.4	—	1.98	25.73	2.98	3.60	0.15	2.74	1.9	1.8
	9	28.93	31.30	19.96	20.66	8.97	10.64	11.81	11.07	8.85	8.87	1.38	23.60	3.92	3.12	0.02	2.30	1.8	1.9
IX	4	34.67	29.92	19.08	18.45	15.59	11.47	11.70	8.66	11.08	7.96	1.04	19.13	2.75	2.47	0.17	2.21	1.9	2.0
	11	41.53	40.51	21.86	20.78	19.67	19.73	9.41	6.10	8.55	9.80	1.12	21.48	4.42	3.24	0.25	2.47	1.9	1.9
	18	46.50	45.34	20.08	19.66	26.42	25.68	10.09	7.99	7.37	8.88	0.89	21.01	3.85	2.91	0.46	2.33	2.0	1.9
X	3	52.82	53.36	38.79	38.59	14.03	14.77	7.47	6.23	7.39	7.39	0.71	19.95	4.00	2.29	0.38	1.87	1.6	1.6
	18	55.44	49.20	46.31	29.84	9.14	19.36	9.94	9.08	4.07	3.53	0.62	15.46	3.56	1.91	0.37	1.78	1.7	1.4
XI	6	59.87	56.06	47.75	36.44	12.12	19.62	9.07	5.73	2.98	2.58	0.70	14.16	1.09	1.67	0.47	2.21	1.2	1.4
	21	68.26	69.50	56.66	69.50	11.60	0.00	5.77	4.26	1.54	1.14	0.63	12.84	1.41	1.53	0.64	1.54	1.2	1.2

TABLE IV.

"Daishiro," in Tanaka.

gramme per fruit.

Date		Total sugar		Reduc. sugar		Sucrose		Pectin		Tannin		Acid	Marc	Water	Wt. per fruit	Remarks
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°					
IX	27	4.78	—	3.68	—	1.10	—	—	—	—	—	—	—	—	69	Becomes yellow.
X	13	10.14	11.91	7.87	8.73	2.27	3.18	2.01	1.27	0.457	0.44	0.116	2.72	71.4	111	acrid taste.
XI	1	16.76	14.97	13.01	10.15	3.75	4.82	1.82	1.02	0.354	0.371	0.132	2.75	97.3	150	
	17	19.54	18.66	19.54	18.66	0.00	0.00	0.75	0.49	0.091	0.052	0.106	3.68	99.6	158	Yellowish orange, softening
XII	4	21.18	21.76	19.74	20.30	1.44	1.26	1.39	1.27	0.178	0.171	0.355	2.37	108.9	169	
	12	23.19	23.18	23.19	20.19	0.00	2.99	1.46	1.03	0.121	0.145	0.175	3.02	116.0	176	pulp, no acrid
	20	25.10	24.14	24.93	20.04	0.95	4.10	1.64	9.85	0.000	0.097	0.189	3.09	127.0	191	taste

TABLE V.

Percentage in "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Marc	Water
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°			
IX	27	10.63	—	6.95	—	3.68	—	—	—	—	—	—	—	—
X	13	14.03	13.00	8.59	9.53	5.44	3.91	2.20	1.29	0.49	0.72	0.12	2.96	78.03
XI	1	14.55	13.08	11.48	8.32	3.07	4.77	1.49	0.83	0.29	0.30	0.11	2.25	79.75
	17	15.48	14.79	15.48	14.79	0.00	0.00	0.64	0.38	0.07	0.05	0.09	2.91	78.93
XII	4	15.51	15.79	14.46	14.87	1.05	0.92	1.01	0.93	0.17	0.12	0.26	1.73	79.06
	12	15.82	15.82	15.82	13.78	0.00	2.04	0.99	1.03	0.08	0.09	0.12	2.06	79.12
	20	15.93	14.85	15.34	12.33	0.59	2.52	1.01	0.85	--	0.05	0.11	1.90	78.24

TABLE VI.

Percentage in dry "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Marc	Glucose-fructo-se-ratio.	
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°			I	II
X	13	63.84	61.18	39.09	43.39	24.75	17.79	10.00	5.86	2.27	3.53	0.57	13.51	1.3	1.2
XI	1	71.81	64.59	56.65	41.05	15.16	23.54	7.37	4.12	1.43	1.50	0.53	11.14	1.1	1.3
	17	73.50	70.18	73.50	70.18	00.00	0.00	3.05	1.82	0.343	0.24	0.40	13.03	1.2	1.2
XII	4	74.12	75.44	69.08	71.03	5.04	4.41	4.85	4.27	0.851	0.59	1.24	8.29	1.1	1.1
	12	75.78	76.39	75.87	61.97	0.00	9.77	4.76	3.64	0.395	0.47	0.56	9.87	1.1	1.2
	20	73.21	66.98	70.50	56.77	2.71	10.21	4.64	3.92	—	0.27	0.53	8.74	1.1	1.2

TABLE VII.

"Marugaki" in the University ground. gm. in per fruit.

Date		Total sugur		Reduc. sugar		Sucrose		Pectin		Tannin		Polysac-charide	Cellulose	Marc	Acid	Nitrogen	Ash	Water	Wt. per fruit	Remarkes
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°									
IX	30	2.97	—	2.49	—	0.48	—	—	—	—	—	—	—	—	—	—	—	—	49	Becomes yellow
X	9	5.21	5.05	4.43	3.71	0.78	1.35	0.59	0.62	0.14	—	0.30	0.20	1.34	0.07	0.03	0.21	37.84	60	
	24	9.09	8.32	8.09	5.50	0.10	2.82	0.94	0.73	0.14	0.20	0.38	0.22	1.79	0.08	0.04	0.27	50.	77	Yellowish orange
XI	13	11.88	8.1	10.4	6.5	0.15	1.6	0.91	0.64	0.13	0.17	0.21	0.34	2.48	0.12	—	0.35	60.00	93	
	28	11.6	11.22	11.6	10.2	0.00	1.02	1.16	0.67	0.11	0.14	0.18	0.24	1.77	0.11	0.08	0.34	57.4	93	Fresh softens, sweet taste.

TABLE VIII.

"Marugaki" in the University ground.

Percentage in "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Polysaccharide	Cellulose	Marc	Acid	Nitrogen	Ash	Water
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°							
IX	30	10.77		9.39		1.4												
X	9	11.11	10.79	9.45	7.92	1.7	2.9	1.3	1.30	0.30		0.6	0.4	2.8	0.15	0.05	0.46	80.95
	24	12.97	12.43	12.82	8.86	0.16	3.6	1.4	1.26	0.31	0.32	0.6	0.3	2.8	0.13	0.06	0.43	80.50
XI	13	13.80	10.67	13.71	8.57	0.20	2.1	1.2	0.84	0.17	0.20	0.2	0.4	3.3	0.16	—	0.46	79.22
	28	14.33	14.28	14.33	12.85	0.00	1.4	1.6	0.94	0.16	0.19	0.2	0.3	2.5	0.16	0.09	0.48	78.44

TABLE IX.

Percentage in dry "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Polysaccharide	Cellulose	Marc	Acid	Nitrogen	Ash	Glucose-fructose-ratio.	
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°							I	II
X	9	58.44	56.67	49.72	41.61	8.72	15.06	6.70	6.95	1.59	—	3.3	2.2	15.0	0.78	0.29	2.4	1.3	1.4
	24	66.54	62.89	65.73	45.44	0.81	17.45	7.64	5.98	1.61	1.63	3.0	1.8	14.5	0.64	0.32	2.1	1.2	1.2
XI	13	66.91	51.38	65.97	41.24	0.94	10.14	5.77	4.37	0.80	0.94	1.3	1.7	15.3	0.75	—	2.6	1.2	1.2
	28	66.49	66.31	66.49	59.69	0.00	6.62	7.55	4.34	0.72	0.88	1.1	1.5	11.5	0.70	0.52	2.2	1.2	1.2

TABLE X.

"Saijo" in Onomichi. gramme per fruit.

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Marc	Water	Wt. per fruit	Remarks
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°					
XI	2	23.72	22.21	19.33	16.32	4.39	5.89	2.55	2.25	0.75	0.43	0.16	4.70	154.4	222	Yellowish orange, acrid taste.
	9	22.68	20.75	22.68	20.75	0.00	0.00	1.41	1.31	0.05	—	0.14	5.38	156.8	197	Sweet.

TABLE XI.

Percentage in "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Marc	Water
		I*	II*	I*	II*	I*	II*	I°	II°	I°	II°			
XI	2	12.61	11.81	10.28	8.68	2.33	3.13	1.36	1.20	0.40	0.23	0.09	2.5	82.15
	9	11.85	10.85	11.85	10.85	0.00	0.00	0.74	0.68	0.02	—	0.07	2.8	81.95

TABLE XII.

Percentage in dry "Pulp".

Date		Total sugar		Reduc. sugar		Cane sugar		Pectin		Tannin		Acid	Mark	Glucose-fructose-ratio	
		I*	II*	I*	II*	I*	II*	I°	II°	I	II°			I	II
II	2	70.65	66.17	57.58	48.61	13.07	17.56	7.60	6.69	2.24	1.27	0.48	14.00	1.2	1.3
	9	65.67	60.10	65.57	60.10	00.00	00.00	4.09	3.79	0.13	—	0.43	15.59	1.1	1.1

II. The Origin of the Reducing Sugar in the Fruit.

The presence of d-fructose, d-glucose and sucrose in the kaki-fruit has been established, yet the relationship of the carbohydrate to one another was undecided.

Studying the fructose-glucose-ratio, during the period of ripening, one of us and Ueda (1923) have, in a previous paper, presented the view that the reducing sugars were formed by hydrolysis from sucrose or some other polysaccharide. To afford some evidence in favour of the above view, the unripe fruit of two strains of astringent variety, and one strain of the sweet, grown at three different places in Kyoto, and picked on Sep. 27 was analysed.

The percentage of total sugar in the fruit picked on the same day, as will be seen in the following table, was fairly constant, no matter what fruit of the different strains was examined. Moreover, the fruit contains much reducing sugar but less sucrose; the quantity of the former in the fruit was in inverse proportion to that of the latter. Such facts render the sucrose theory concerning the origin of the reducing sugars during the period of growth, although conclusive evidence on this point is lacking.

	"Marugaki" Tanaka.	"Marugaki" University ground	"Daishiro" Tanaka.
	%	%	%
Total sugar	12.4	10.8	10.6
Reducing sugar	10.4	8.5	7.0
Sucrose	2.0	2.3	3.4
Fructose-glucose-ratio	1.39	1.36	1.53
Weight per fruit	70 gm.	59 gm.	29 gm.

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ÜBER DIE GLYKOLYTISCHE KRAFT DES BLUTES.

II. Mitteilung.

Von

YOSHIKANE KAWASHIMA.

(*Aus der biochemischen Abteilung des Institutes für Infektionskrankheiten der Universität zu Tokyo. Vorstand: Dr. Teisuke Kohmoto.*)

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I. Einleitung.

Aus der vorigen Mitteilung (1922) stehen wir nunmehr zweifellos vor dem Schlusse, dass die glykolytische Kraft der Erythrozyten mit ihrer Lebensaktivität eng verknüpft ist. Sie geht nämlich schon durch den einfachen mechanischen Zerfall der Zellen, oder doch durch eintägiges Belassen der Blutkörperchenaufschwemmung im Brutschrank verloren, während die Blutkörperchen dabei in der Form noch gut erhalten sind.

Hier möchte der Verfasser zur Beifügung noch die Ergebnisse einiger weiterer Experimente berichten, die das Resultat der vorigen Experimente bestätigen.

II. Untersuchungsmethode.

Die Untersuchungsmethode folgt im grossen und ganzen auch der bei den früheren Untersuchungen. Aber über verschiedene Punkte ist hier einiges zu bemerken. Als Versuchstier standen uns Kaninchen zur Verfügung. Das entnommene Blut wurde sofort defibriniert, und zu diesem, unter Umständen nach einiger Behandlung, eine geringe Menge der isotonischen Kochsalzlösung mit 10%igem Traubenzucker hinzugefügt, um den Ausschlag des Versuchs deutlich erfolgen zu lassen. Zur Zuckerbestimmung

bediente ich mich des Mikroverfahrens von Bang. Natürlich wurde alles unter streng aseptischen Kauteln ausgeführt, während die Bakterienfreiheit jeder Probe stets durch aerobe und anaerobe Kultur sichergestellt wurde.

III. Die glykolytische Kraft der Erythrozyten in der hypotonischen Kochsalzlösung.

Aus der vorigen Mitteilung ist ersichtlich, dass die glykolytische Kraft der Erythrozyten in der hypertonischen Kochsalzlösung fast immer unverändert bleibt, selbst wenn ihre Konzentration 1 Mol. oder noch darüber hinaufgeht. So glaube ich dass es von Interesse sei zu prüfen, wie die glykolytische Kraft der Erythrozyten in der hypotonischen Kochsalzlösung sich verhält, in welcher die Zellen eine deutlich formliche und funktionelle Veränderung unter der Aufquellung erfahren.

TABELLE I.

Versuchsfälle	Gehalt von NaCl (%)	Zuckergehalt in %			Zuckerabnahme in		Zahl der Erythrozyt. Einheit, Tausend	Relative Zuckerabnahme zu 1 Mil. Erythr.
		Vor dem Versuch	Nach 5 St. in 37°C.	Nach 24 St. in 37°C.	mg.	%		
I. Kontrollvers.	0.85	0.240		0.136	104	43.3	6,161	16.9
Eigentl. Vers.	0.4	0.294		0.279	15	5.1	80(?)	
II. Kontrollvers.	„	0.221		0.092	129	58.4	6,187 (±) 5,759	20.9
Eigentl. Vers.	0.5	0.199		0.114	85	42.7	(4,747) 17.6%	14.8
III. Kontrollvers.	„	0.220	0.169	0.129	51–91	23.2–41.4	6,264 (5,129) 18.1%	8.1–14.5
Eigentl. Vers.	0.5	0.287	0.263	0.249	24–38	8.4–13.2	2,466(?) (66) 97.3%	9.7–15.4
IV. Kontrollvers.	„	0.247	0.188	0.119	59–128	23.9–51.8	6,011 (5,913) 1.6%	9.8–21.3
Eigentl. Vers.	0.5	0.229	0.195	0.136	34–93	14.8–40.6	5,753 (3,839) 33.3%	5.9–16.2

(?) = Mikroskopisch sind eine nicht so geringe Anzahl Erythrozyten zu erkennen, z. T. zerfallend, z. T. noch gut geformt, meistens mit Stroma sich zusammenklumpend. Die angegebene Zahl ist also keine sichere.

(?′) = Bei der Zählung sind etwa vorhandene Schatten mitberechnet

Die Erythrozytenzahl in den Klammern wurde am Ende der Prüfung des folgenden Tages berechnet, und die Prozentzahl zeigt eine abgenommene Proportion der Erythrozyten, welche während der Versuchsdauer zu grunde gingen.

(±) = Kein Zahlenunterschied vor und nach dem Versuch.

Aus der Tabelle I kann man ersehen, dass die glykolytische Kraft der Blutkörperchen in der hypotonischen Kochsalzlösung in einem parallelen Verhältnis mit ihrem Zerfall steht, ebensowie die in der hypertonischen. Dieses Verhalten war ausgeprägt bei dem Versuch I, wobei die Blutkörperchen schon während des dreimaligen Auswaschens mit der 0.4%igen Kochsalzlösung grösstenteils zerfielen und auch die glykolytische Kraft auf ein Minimum zurückging. In den Fällen II und IV, wo der Zerfall beim Auswaschen nicht so deutlich war, und erst während der Versuchsdauer einen ziemlich starken Grad erreichte, zeigt die Zuckerabnahme nur etwas geringeren Wert als die Kontrollen, was als Folge der verschwindenden Erythrozyten leicht verständlich ist. Jedenfalls kann man sagen, dass die glykolytische Kraft der Erythrozyten in der hypotonischen Kochsalzlösung fast unbeeinträchtigt bleibt, solange die Erythrozyten darin noch nicht vollständiger Zerstörung anheimfallen, wenn sie auch schon aufgequollen und gewissermassen geschädigt sind.

IV. Versuch mit dem vom Serum möglichst befreiten durch Frieren und Auftauen hämolysierten Erythrozytenbreie.

Bei diesem Versuche wurde das entnommene defibrinierte Kaninchenblut auf eine kräftige Zentrifuge gebracht, und das abgeschiedene Serum möglichst genügend abpipettiert. Ein Teil von solchem Blutzellenbrei wurde als Kontrollprobe genommen, der andere Teil davon durch wiederholtes Frieren und Auftauen

hämolysiert (Eintauchen in ein Gemisch von Eis und Kochsalz, dann in warmes Wasser von ca. 38–40°C). Dann wurden die beiden Proben mit der 10%igen Traubenzuckerlösung von einer gewissen Menge versetzt und zur Untersuchung angestellt. Während des Hämolysierungsverfahrens wurde der Blutzellenbrei oft kräftig zentrifugiert, um die zurückgebliebenen geformten Elemente möglichst zu entfernen, und dann mit der Zuckerlösung versetzt, um die Glykolyse zu untersuchen. (Tabelle II eigentl. Versuche B. D. E.)

TABELLE II.

Versuchsfälle		Zuckergehalt in %		Zuckerabnahme in		Zahl der Erythrozyt. Einheit, Tausend.	Relative Zuckerabnahme zu 1 Mil. Erythrozyt.
		Vor dem Versuch	Nach 20–23 St. in 37°C.	mg.	%		
Kontrollversuch		0.345	0.071	274	79.4	17,005 (8,702) 48.8%	16.1
Eigentl. Vers.	A (20X)^	0.387	0.372	15	3.9	59(?)	
	B (10X)*	0.237	0.252	0	0	0	
Kontrollversuch		0.479	0.149	330	68.9	15,476 (3,105) 79.9%	21.3
Eigentl. Vers.	C (10X)^	0.557	0.528	29	5.2	134(?)	
	D (5X)*	0.549	0.527	22	4.0	17(?)	
Eigentl. Vers.	E (2X)*	0.306	0.315	0	0	0	

^ nicht zentrifugiert.

* zentrifugiert.

(20X) (10X) etc. = wiederholte Zahl des Hämolysierungsverfahrens.

Erythrozytenzahl mit (?) ist nicht ganz sicher, weil die Zellen in einer schleimig-zähen Probematerie nicht ganz gleichmässig verteilt sind.

Wie die Tabelle II zeigt, liess sich noch eine ganz geringe Zuckerabnahme bei der Zerstörung der Erythrozyten erkennen

(Vers. A. u. C.), welche natürlich auf der Aktivität der vom Zerfall verschont gebliebenen Erythrozyten beruhen muss, da die durch die Zentrifugierung zellenfrei gemachte Flüssigkeit keine glykolytische Kraft mehr hatte (Vers. B. u. E.). Der Versuch E wurde möglichst rasch nach dem zweimaligen Zerfallungsverfahren angestellt, damit die Frage festgestellt wird, ob die glykolytische Kraft sich schon während des mehrmaligen langen Verfahrens verlieren werde. Beim Versuche D konnte zellenfreie Materie trotz des kräftigen Zentrifugieren nicht gewonnen werden; sie zeigt eine sehr schwache Glykolyse wie die beim Versuch A. u. C., wobei eine vollständige Hämolyse selbst nach 10–20 mal wiederholtem Zerfallungsverfahren nicht herbeizuführen war.

Daraus ist es hier klar gestellt, dass die glykolytische Kraft der Erythrozyten mit ihrem Zerfall verschwindet, denn die hämolysierte Substanz aus den vom Serum befreiten Blutkörperchen, deren Inhalt wahrscheinlich ein geeigneteres Medium für den glykolytischen Vorgang sein mag, zeigt keine glykolytische Kraft mehr. Wie aus der Tabelle II ersichtlich ist, verrichten die unzerstörten widerständigen Erythrozyten eine relativ grössere Zuckerspaltung, worauf ich im nächsten Experimente noch weiter eingehen will.

V. Vergleichende Versuche mit dem Erythrozytenbrei und Vollblut u. s. w.

TABELLE III.

	Versuchsfälle	Zuckergehalt in %		Zuckerabnahme in		Zahl der Erythrozyt. Einheit, Tausend.	Relative Zuckerabnahme zu 1 Mil. Erythrozyt.
		Vor dem Versuch	Nach 22–23 St. in 37°C.	mg.	%		
I.	Vollblut + Zucker	0.272	0.056	216	79.4	6,821	31.7
	B. Z. B + Zucker	0.491	0.155	336	68.4	13,171	25.5
	Desgleichen + O. H. Z. (aa.)	0.302	0.070	232	76.8	7,221	32.1
	O. H. Z. + Zucker	0.257	0.257	0	0	Mikroskopisch ganz geringe Anzahl Erythrozyten sichtbar.	

	Versuchsfälle	Zuckergehalt in %		Zuckerabnahme in		Zahl der Erythrozyt. Einheit, Tausend.	Relative Zuckerabnahme zu 1 Mil. Erythrozyt.
		Vor dem Versuch	Nach 22–23 St. in 37°C.	mg.	%		
II.	a. Vollblut + Zucker	0.274	0.059	215	78.5	7,456	28.8
	b. B. Z. B. + Serum (ca. 9 mal mehr Vol.) + Zucker	0.256	0.245	111	31.2	2,343	47.4
	c. B. Z. B. + O. H. Z. (aa.) + Zucker	0.273	0.041	232	85.0	9,314	24.9
III.	Vollblut + Zucker	0.366	0.143	223	60.9	6,915	32.2
	B. Z. B. + Serum (ca. 19 mal mehr Vol.) + Zucker	0.456	0.410	46	10.1	0,608	75.5
IV.	a. B. Z. B. + Serum (aa.) + Zucker	0.424	0.212	212	50.0	7,448	28.5
	b. Desgleichen (ca. 9 mal mehr Serum.)	0.484	0.431	53	11.0	1,248	42.5
	c. B. Z. B. + O. H. Z. (aa.) + Zucker	0.345	0.179	166	48.1	6,536	25.4
	d. Desgleichen (ca. 9 mal mehr Vol. O. H. Z.)	0.319	0.245	74	23.2	1,240	59.7
	e. Gewaschene Blutzellen + NaCl-lösg. (aa.) + Zucker	0.364	0.251	113	31.0	5,920	19.1
	f. Desgleichen (ca. 9 mal mehr Vol. NaCl-lösung)	0.335	0.315	20	6.0	1,688	11.8

B. Z. B. = Der vom Serum möglichst befreite Blutzellenbrei unter Anwendung der Zentrifuge.

O. H. Z. = Obere hämolysierte Zentrifugalmaterie, welche nach der Hämolyse des obigen Blutzellenbreis durch Frieren und Auftauen abgetrennt wurde.

Bei diesen Versuchen wurde erstens die glykolytische Kraft des Vollblutes mit der von dem Blutzellenbrei allein, oder mit der von den in der oberen hämolysierten Flüssigkeit suspendierten Blutkörperchen, d. h. den im Zellinhalt aufgeschwemmten Blutkörperchen, verglichen. Zweitens wurden die zwei Proben unter

gleichen Bedingungen hergestellt, deren eine die fast normale Anzahl von Erythrozyten, eine andere nur eine geringe Anzahl davon enthält, um in ihrer glykolytischen Kraft miteinander kollationiert zu werden.

Wie aus der Tabelle III veranschaulicht ist, zeigen das Vollblut und der Blutzellenbrei mit der hämolysierten oberen Zentrifugalflüssigkeit in ihrer glykolytischen Kraft einen fast gleichen Wert, während beim Blutzellenbrei allein ein niedrigerer als diese beiden sich erkennen lässt. Beim letzteren nähert sich die abgenommene Zuckermenge zu I Mil. Erythrozyten derselben bei den Erythrozyten in physiologischer Kochsalzlösung (siehe auch die Tabelle II Kontrollversuch). Wenn man die Erythrozytenzahl in der Probe geringer macht, so tritt die Glykolyse in einem stärkeren Masse als die beim fast normalen Gehalt von Erythrozyten ein: wie die Versuchsfälle II a. b., III u. IV a. b. u. c. d. zeigen, wurde beim Gehalt von 2343 und 1248 Tausend Erythrozyten mit Serum 1.6–1.5 fache, von 608 Tausend 2.3 fache, und von 1240 Tausend Erythrozyten mit der oberen hämolysierten Zentrifugalflüssigkeit eine 2.4 fach grössere Zuckermenge im Verhältnisse zu 1 Mil. Erythrozyten als die Kontrollen abgespalten. Bei den Erythrozyten in der physiologischen Kochsalzlösung, den letzten Versuchen e. u. f. in der Tabelle III, ist dies aber nicht der Fall, sondern es ist eine etwas kleinere Zuckerabnahme beim geringen Erythrozytengehalt zu erkennen, worüber die genaue Beschreibung sich in meiner ersten Mitteilung findet.

Aus den obigen Ergebnissen kann man sagen, dass das Serum und die oben hämolysierte Zentrifugalmaterie, der gelöste Zellinhalt, ein passendes Medium vom fast gleichgradigen Erfolg zur glykolytischen Wirkung der Erythrozyten bieten. Bei den Erythrozyten ohne das Medium von einer bestimmten Menge schwächt sich ihre glykolytische Kraft ab, was in den Versuchsfällen mit dem Erythrozytenbrei zu sehen ist. Wenn dagegen das Medium an Menge im Verhältnis zu den Erythrozyten vermehrt ist, so ist ihre glykolytische Kraft stärker entfaltet,

abgesehen vom Falle mit der physiologischen Kochsalzlösung. Natürlich kommt der glykolytische Vorgang auch nicht in Erscheinung, wenn die Zahl der Erythrozyten in der Probe sehr stark abnimmt. Das Phosphatgemisch von Blutalkaleszenz ist auch für die Glykolyse der Erythrozyten vom gleichen Verhalten mit dem Serum oder dem gelösten Zellinhalt, was aus der Tabelle IV u. V in der vorigen Mitteilung verständlich ist. Nachstehende Tabelle zeigt wieder die kurzgefassten Resultate.

TABELLE IV.

Versuchsfälle	Zuckergehalt in %		Zucker-abnahme in		Zahl der Erythrozyt. Einheit, Tausend	Relative Zuckerabnahme zu 1 Mil. Erythrozyt.
	Vor dem Versuch	Nach 21–24 St. in 37°C.	mg.	%		
Vollblut+Zucker	0.274	0.076	198	72.3	5,840	33.9
Ausgewaschene Blutzellen +Phosphatgemisch von Blutalkaleszenz+Zucker	0.299	0.106	193	64.5	5,224	36.9
Desgleichen	0.228	0.139	89	39.0	1,588	56.0

Endlich kann man auch vermuten, dass der gelöste Zellinhalt nicht mehr glykolytisch wirkt und bei dem Versuche nur als eine Neutralisationsmaterie der Zuckerspaltungsprodukte wirkt wie das Serum oder Phosphatgemisch, um die glykolytische Aktivität der Erythrozyten lang und stark vor sich gehen zu lassen. Also ist es auch leicht erklärlich, dass die Erythrozyten stärker aktiv sind, wenn sie in einer grösseren Menge von Serum oder Phosphatgemisch aufgeschwemmt sind.

VI. Schlussfolgerungen.

I) In der hypotonischen Kochsalzlösung wird die glykolytische Kraft der Erythrozyten nicht so deutlich verändert, solange diese nicht zur Hämolyse kommen.

II) Mit der Hämolyse des Blutzellenbreis durch Frieren und Auftauen geht auch die glykolytische Kraft verloren.

III) Die Aufschwemmung der Blutkörperchen im durch Hämolyse gewonnenen Zellinhalt wirkt fast gleich stark glykolytisch wie das Vollblut; vorausgesetzt, dass die Blutkörperchen in einem Medium von einer fast gleichen Menge aufgeschwemmt sind.

IV) Die Blutkörperchen zeigen eine stärkere glykolytische Kraft, wenn sie in einer grösseren Menge von Serum, Phosphatgemisch bzw. Erythrozyteninhalt, nicht aber Kochsalzlösung, aufgeschwemmt sind; die Ursache liegt wahrscheinlich darin, dass die Medien auf die Produkte der Glykolyse neutralisierend wirken.

V) Der gelöste Zellinhalt zeigt keine glykolytische Kraft.

VI) Die Erythrozyten wirken glykolytisch, solange sie nicht nur morphologisch sondern auch funktionell aktiv sind. Wenn sie zum Zerfall kommen oder ihre lebendige Kraft verlieren, obgleich sie mikroskopisch in der Form noch gut erhalten sind, so kommt auch ihre glykolytische Kraft zum Verschwinden.

Im Anschluss daran führte der Verfasser viele Experimente über die Beziehung zwischen der glykolytischen Kraft und der Atmung der Erythrozyten aus, deren Ergebnisse bald nachfolgend als dritte Mitteilung erscheinen sollen.

LITERATUR

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